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Organic residue analysis of hunter-gatherer pottery from Zengpiyan, China, to investigate vessel use and diet

Organic residue analysis of hunter-gatherer pottery from Zengpiyan, China, to investigate vessel use and diet

by

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Abstract

The chemical analysis of organic residues associated with archaeological artefacts can help to unravel ancient diet, food habits, trade and economy. Organic residue analysis (ORA) of archaeological ceramics targets preserved biomolecules originating from processing foodstuffs. Lipids can be separated and characterised as individual molecular species by a suite of analytical techniques. By applying the archaeological biomarker concept, we can assign biomolecular components to specific animal and plant sources based on well-established biomarker criteria.

Zengpiyan is an early Neolithic site located in southern China, occupied between 12,000 and 7,500 B.P. Subsistence activities are defined from the zooarchaeological and archaeobotanical remains investigated thus far, with hunting and gathering seems to be the major subsistence strategies for food procurement. Freshwater shellfish and wild deer bones dominated in the total faunal assemblage, being the staple foods. No trace of any rice cultivation existed, and previous investigation of plant exploitation provided only limited information. This project applied ORA to investigate the subsistence practices in Zengpiyan, in combination with archaeological evidence to test pre-existing hypothesizes and develop a more comprehensive picture of the subsistence practices of the inhabitants of this site.

A total of 58 archaeological potsherds from different cultural phases of Zengpiyan were analysed, using both the acidified methanol and solvent extraction methods. The potsherds exhibited a recovery rate of about 33% with a mean lipid concentration of 0.22 mg g^{-1}. Ten total lipid extracts were dominated by degraded animal fats, $C_{16:0}$ and $C_{18:0}$ fatty acids, indicating the processing of animal products. The $\delta^{13}C$ values of the major fatty acids determined the sources as ruminant adipose and non-ruminant adipose fats, which correlated with the dominance of deer and pig bones recovered.

Novel long-chain fatty acid distributions were observed, together with other plant biomarkers, i.e. *n*-alcohols, *n*-alkanes and diterpenoids, although these were only present in low abundance. Although dihydroxy fatty acids, APAAs and isoprenoids were detected in 2 extracts their compositions could not confirm the processing of aquatic products in the vessels. The wide range of $\delta^{13}C$ values observed for the animal fats suggest the animals foraged on a mixture of C_3 and C_4 plants, possibly due seasonal migrations or a mixed local plant ecosystem.

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Finally, I'd like to give special thanks to my parents and friends, for their constant encouragement and support over the last two years. Thanks to Xiaoyang, for accompanying me over the last few months. A very special thanks to my parents encouraging me to do the thing I want to do and providing me with unwavering moral and emotional support.

Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the requirement of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, other, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED DATE

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List of abbreviations

ACL	Average chain lengths
B.P.	Before the present
BSTFA	*N,O*-bis(trimethylsilyl)trifluoroacetamide
CAM	Crassulacean acid metabolism
CPI	Carbon preference index
DAG	Diacylglycerol
DCM	Dichloromethane
DHYA	Dihydroxy fatty acid
EI	Electron ionisation
FAME	Fatty acid methyl ester
FID	Flame ionization detector
GC	Gas chromatography
GC-C-IRMS	Gas chromatography-combustion-isotope ratio mass spectrometry
GC-MS	Gas chromatography-mass spectrometry
HTGC	High-temperature gas chromatography
IS	Internal standard
LCFA	Long-chain fatty acid
MAG	Monoacylglycerol
MS	Mass spectrometry
NIST	National Institute of Standards and Technology
ORA	Organic residue analysis
PGA	3-phosphoglycerate
PUFA	Polyunsaturated fatty acid
SIM	Selected ion monitoring
TAG	Triacylglycerol
TLE	Total lipid extract
TMS	Trimethylsilyl

1. Literature review

1.1 Organic residue analysis in archaeological pottery

Pottery vessels are one of the most widely excavated materials from archaeological sites, commonly found as fragments in the form of potsherds. These thermally-resistant artifacts are durable and regarded as one of the most important archaeological finds. Traditional methods of analysing pottery have mainly focused on the study of vessels forms and manufacturing techniques to interpret functions and chronological relationship (Tite, 2008; Rice, 2015). Organic residue analysis has revealed lipids (Fig. 1-1), surviving in >80% cooking pottery assemblages worldwide, are absorbed into unglazed ceramic pottery during processing foodstuffs (Evershed, 2008). Their entrapments within the porous ceramic walls and inherent hydrophobicity limits loss, and high saturated structures limits alteration or degradation over time.Hence, these biomolecules have the capacity of providing information on what kind of commodities were processed by ancient people within pottery vessels, and hence prehistoric diet and subsistence strategies.

Investigation of lipid residues absorbed within the pottery vessels is mainly concerned with analysing lipids *via* gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), high temperature gas chromatography (HTGC) and HTGC-mass spectrometry (HTGC-MS) and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS; Evershed *et al.,* 1990; Evershed, 1993, Evershed *et al.,* 1994; Evershed, 2008). These techniques allow separation and identification of the lipid components of extracts, and determination of stable isotope values. Such information forms the basis for assigning specific sources of commodities contributing to organic residues surviving within pottery vessels. Based on combined molecular and isotopic approach a wide range of commodities have been detected in pottery vessels, including terrestrial animal products (e.g. Evershed *et al.,* 1991a, 1994, 2002b; Mottram *et al.,* 1999; Coley *et al.,* 2003; Regert, 2011), aquatic commodities (e.g. Hansel *et al.,* 2004; Evershed *et al.,* 2008a; Cramp *et al.,* 2014b), plant waxes (e.g. Evershed *et al.,* 1991b; Reber and Evershed, 2004a, b; Reber *et al.,* 2004; Cramp *et al.,* 2011; Dunne *et al.,* 2017), beeswax (e.g. Evershed *et al.,* 1997c; Regert *et al.,* 2001; Kimpe *et al.,* 2002; Roffet-Salque *et al.,* 2013, 2015), resins (e.g. Evershed *et al.,* 1985, 1997b; Mills and White, 1989; Stern *et al.,* 2003; Regert *et al.,* 2008), petroleum bitumen (e.g. Connan *et al.,* 2004), birch bark tar (e.g. Charters *et al.,* 1993; Urem-Kotsou *et al.,* 2002; Lucquin *et al.,* 2007), etc.

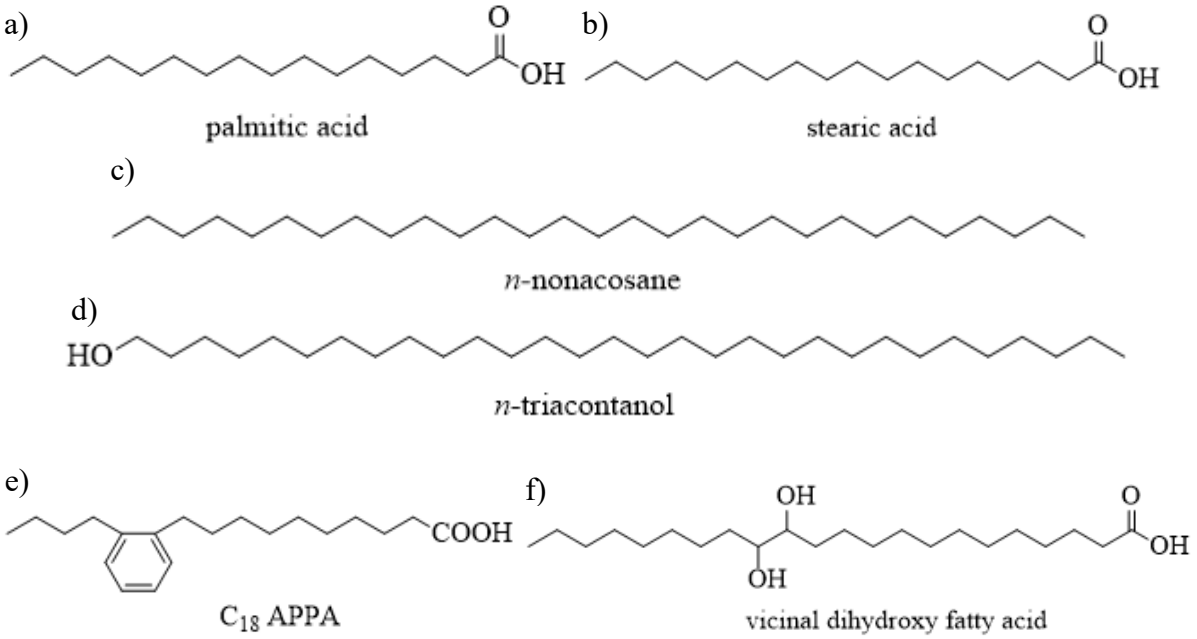


Figure 1-1 Representative lipid biomarkers detected within archaeological potsherds including a) and b) fatty acids, $C_{16:0}$ and $C_{18:0}$, characteristic of degraded animal fats, c) and d) C_{29} *n*-alkane and C_{30} *n*-alcohol, indicator of epicuticular plant waxes, e) and f) C_{18} ω-(*o*-alkylphenyl)alkanoic acids (APAA) and 9,10-dihydroxystearic fatty acid, characteristic of aquatic fats. (Evershed, 2008a).

1.2 Archaeological biomarkers

1.2.1 The identification of animal fats

As previously stated, lipids originating from degraded animal fats have been detected from pottery excavated across the world including Europe (Dudd *et al.,* 1999; Copley *et al.,* 2005; Roffet-Salque *et al.,* 2013; Whelton *et al.,* 2018), Africa (Dunne *et al.,* 2012, 2018), the Near East (Evershed *et al.,* 2008b), America (Eerkens, 2001, 2005) and East Asia (Craig *et al.,* 2013; Lucquin *et al.,* 2016b). Triacylglycerols (TAGs) are the major components of fresh animal fats. Specific TAG distributions can be diagnostic of different animal sources, i.e. non-ruminant adipose, ruminant adipose, or ruminant dairy fats, however, extensive degradation during vessel use and burial can limit the use of TAGs for determining the original of ancient fats (Dudd and Evershed, 1998; Mottram *et al.,* 1999; Evershed *et al.,* 2002b; Copley *et al.,* 2003). TAGs present in animal adipose fats contain C_{48} to C_{54} acyl carbon atoms, with C_{50} and C_{52} dominating (Evershed *et al.,* 2002b), while in dairy fats, the number of acyl carbon atoms varies from 40 to 52 (Dudd and Evershed, 1998). Distinguishing dairy fats from degraded ruminant carcass fats based on TAGs is rarely achieved as the ester bonds are easily cleaved during vessel use and burial *via* enzymatic or chemical hydrolysis to diacylglycerols (DAGs), monoacylglycerols (MAGs) and free fatty acids (Fig.1-2 ; Evershed *et al.,* 1995; Dudd *et al.,* 1998).

Despite the TAGs being lost by degradation, the origin of ancient animal fats can be determined based on their fatty acid compositions, and double bond positions (Evershed *et al.,* 1997a; Mottram *et al.,* 1999). Degraded animal fats present are identified by the high abundance of palmitic acid (*n*-C_{16} fatty acid) and stearic acid (*n*-C_{18} fatty acid; Fig. 1-1; Evershed *et al*., 1997a; Evershed *et al*., 2002b). The stable carbon isotope ($\delta^{13}C$) values of *n*-$C_{16:0}$ and *n*-$C_{18:0}$ fatty acids are of particular value in assigning the origins of remnant fatty acids derived from ruminant or non-ruminant animal and dairy products and will be discussed in detail below (Dudd *et al.,* 1999; Copley *et al.,* 2003). The observation of odd-chain and branched-chain fatty acids, C_{15} and C_{17} are indicative bacterial populations in ruminant animals (Dudd and Evershed, 1998).

1.2.2 $\delta^{13}C$ values of animal fats

Although degraded animal fats can be readily detected from archaeological potsherds, determining their origins or specifying whether they are mixtures of fats of different species is quite problematic. The $\delta^{13}C$ values of fatty acids from different animal species, i.e. ruminant adipose (e.g. sheep, cattle and goat), ruminant dairy products (e.g. milk and cheese), non-ruminant adipose (e.g. porcine), and aquatic animals, varies by differential routing of dietary carbon and fatty acids during synthesis of adipose and dairy fats (Dudd and Evershed, 1998; Copley *et al.,* 2003; Cramp and Evershed, 2014). Assigning the origins of recovered lipid residues to specific animal sources relies on the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids in tandem with the $\Delta^{13}C$ ($\delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) values. Analyses of modern reference fats has shown that ruminant adipose fats are more depleted than non-ruminant adipose fats, in $C_{16:0}$ and $C_{18:0}$ fatty acids by 3.8‰ and 6.9‰, respectively (Fig. 1-3; Copley *et al.,* 2003).

Dairy products (e.g. cheese or milk) have been shown to be an important element of diet in prehistoric Europe (Sherratt, 1981; Barker, 1985) based om fats detected in pottery vessels from Europe (Dudd and Evershed, 1998; Roffet-Salque *et al.,* 2013), Africa (Dunne *et al.,* 2012) and the Near East (Evershed *et al.,* 2008b). While fresh dairy fats are characterised by short-chain fatty acids (*n*-$C_{4:0}$ to *n*-$C_{12:0}$), these diagnostic lipids are rarely preserved in ancient pottery vessels over long archaeological timescales. Short-chain fatty acids are water-soluble and easy to hydrolyse during vessel uses and burial. Hence, the use of compound-specific stable carbon isotope values is essential for distinguishing the adipose and milk fats in pottery lipid residues and investigating the origins of dairying in antiquity (Dudd and Evershed, 1998; Evershed *et al.,* 2002b; Copley *et al.,* 2003).

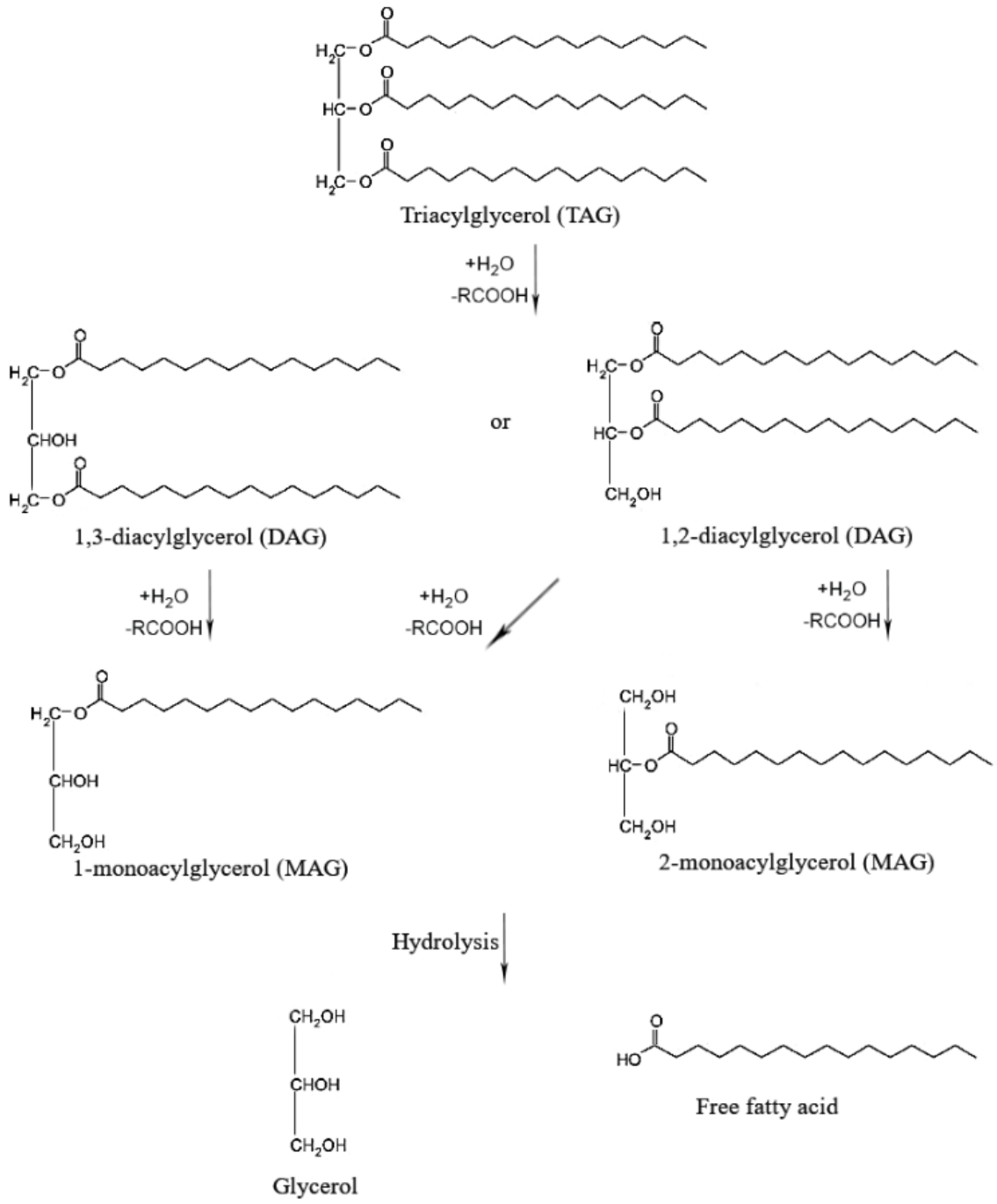


Figure 1-2 Hydrolytic pathway for the degradation of TAGs to DAGs, MAGs and free fatty acids in animals. (adapted from Evershed *et al.,* 2002b).

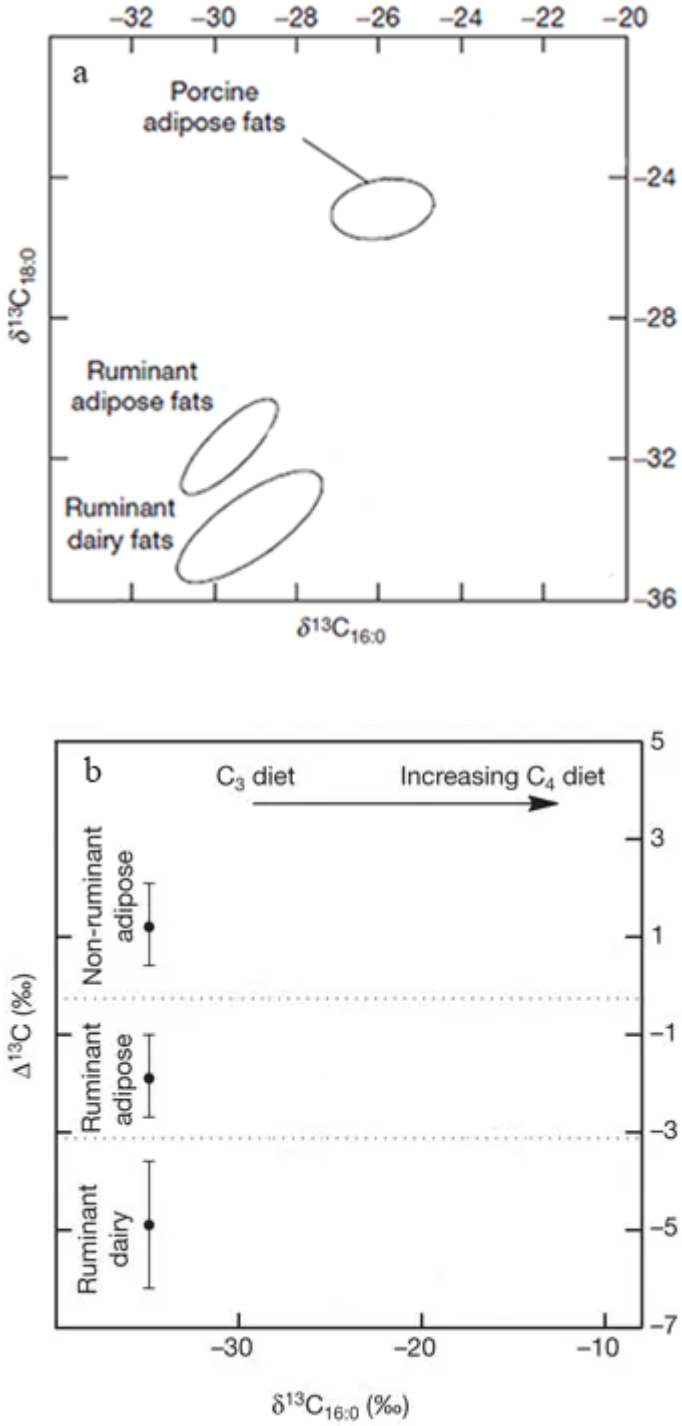


Figure 1-3 a). Isotopic proxies for the major fatty acids, $C_{16:0}$ and $C_{18:0}$, derived from non-ruminant (porcine) adipose, ruminant adipose and ruminant dairy fats based on modern reference fats from animals raised on a strict C_3 diet in Britain b). Plot of $\Delta^{13}C$ ($\delta^{13}C_{18:0}$ - $\delta^{13}C_{16:0}$) values plotted against $\delta^{13}C_{16:0}$. The $\delta^{13}C$ values of modern reference fats were adjusted for post-Industrial Revolution effects of fossil fuel burning by the addition of 1.2‰ (Friedl *et al.,* 1986). The confidence ellipses represent the $\delta^{13}C$ values of modern reference animals in Britain (raised in a pure C_3 diet), Africa, Kazakhstan, Switzerland and the Near East. Analytical precision is ±0.3‰ (Copley *et al.,* 2003; Dunne *et al.*, 2012).

The carbon isotope discrimination of adipose and milk fats is based on differences in their biosynthesis within the animals. In ruminant animals the $C_{16:0}$ and $C_{18:0}$ fatty acids in adipose tissues are primarily biosynthesised *de novo* from acetate (derived from dietary carbohydrate). However, for dairy fats the situation is more complex, with the mammary gland only having the capacity to biosynthesis $C_{16:0}$ component *de novo* from acetate (Moore and Christie, 1981; Byers and Schelling, 1988). Hence, the $C_{18:0}$ fatty acid required for milk fat biosynthesis must be obtained from elsewhere. About 60% of the $C_{18:0}$ fatty acid is sourced from the mono, di-, and triunsaturated C_{18} fatty acids derived from the plant diet, following biohydrogenation in the rumen, with the remainder (40%) coming from adipose derived *n*-$C_{18:0}$ fatty acids. The $\delta^{13}C$ values of unsaturated fatty acids in plants is about 8.1‰ less than carbohydrates (DeNiro and Epstein, 1977), leading to a 2.3‰ depletion exhibited by the $\delta^{13}C_{18:0}$ value of dairy fats compared to adipose fats in ruminant animals (Fig. 1-4; Dudd and Evershed, 1998; Copley *et al.,* 2003).

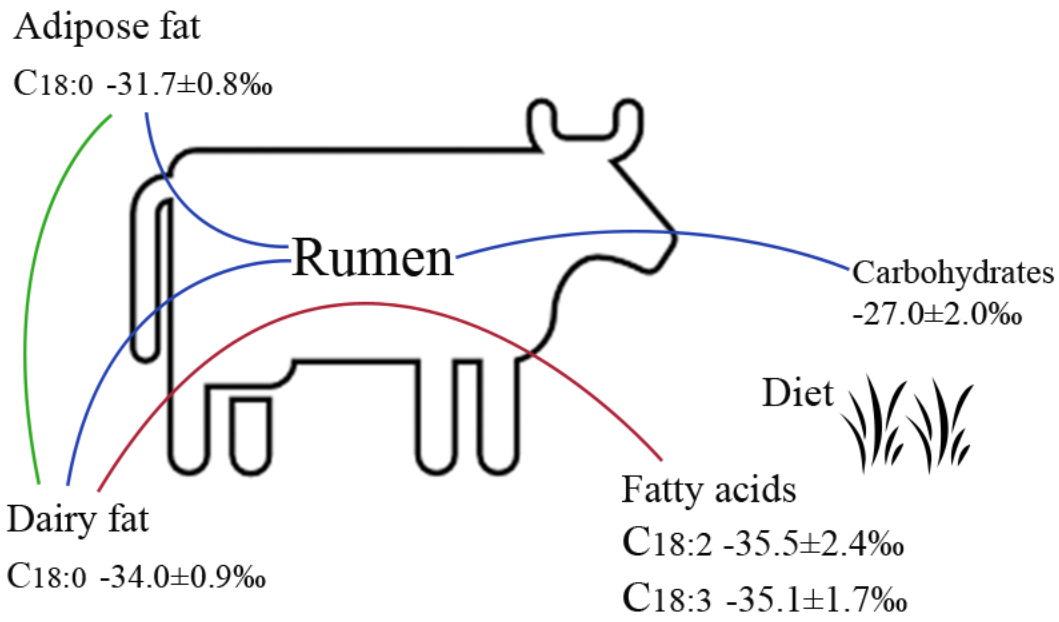


Figure 1-4 Diagram showing the differential routing of carbon precursors during the biosynthesising of fatty acid $C_{18:0}$ in ruminant animals (adapted from Copley *et al.,* 2003).

Animal products processed in pots will display characteristic isotopic signatures of the ecosystems the animal lived in (Gannes *et al.,* 1997), i.e. animals with a diet comprising C_4/marine resources will have lighter $\delta^{13}C$ values than those raised solely on terrestrial C_3 plants (Roffet-Salque, 2012). Thus, the $\delta^{13}C$ values of fatty acids extracted from potsherds will be a direct reflection of the animal pastures, providing information about the environment that animal foraged in. The $\Delta^{13}C$ value ($\delta^{13}C_{18:0}$-$\delta^{13}C_{16:0}$) is a recognized criterion for eliminating

the isotopic influence from the living environment to distinguish lipid origins, e.g. non-ruminant adipose, ruminant adipose and dairy fats (Evershed *et al.,* 1997a; Dudd and Evershed, 1998; Dudd *et al.,* 1999; Evershed *et al.,* 2008a). The exogenous influence from the environment affects both $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ equally. The $\Delta^{13}C$ value plotted against $\delta^{13}C_{16:0}$, reflects the metabolism differences between animal species and the environment input simultaneously (Evershed *et al.,* 2008a; Dunne *et al.,* 2012; Roffet-Salque, 2012).

Despite the domesticated animals, wild animals like deer, or aquatic (marine or freshwater) exhibit different isotope signature (Dufour *et al.,* 1999; Craig *et al.,* 2012; Cramp and Evershed, 2014). Fat originating from marine organism have less depleted $\delta^{13}C$ values when compared with the terrestrial fats (Cramp and Evershed, 2014; Pääkkönen *et al.,* 2016, 2018). The carbon source of freshwater commodities comes from various origins, e.g. dissolved inorganic carbon or degraded organic matter, thus exhibiting relative variable isotope composition and overlap ruminant and non-ruminant adipose fats, or even the C_3 plant range (Dufour *et al.,* 1999). Wild ruminant animals, deer, have been hunted in China as a staple dietary food in ancient time (Chinese Academy of Social Science *et al.,* 2003; Liu and Chen, 2012). Due to their flexible habitat compositions, the carbon isotope values even reach the range of dairy fats (Craig *et al.,* 2012).

1.2.3 The identification of plant lipids

1.2.3.1 Plant photosynthetic pathways

There are three metabolic pathways for carbon fixation used by plants in photosynthesis, C_3, C_4 and Crassulacean acid metabolism (CAM; Fig. 1-5). Plants that use C_3 carbon fixation (or the Calvin cycle) utilize the enzyme, ribulose-1,5-biphosphate carboxylase (RuBisCo), to convert atmospheric CO_2 into 3-phosphoglycerate (PGA) (Calvin and Benson, 1948). C_3 plants comprise up to 90% of terrestrial plants worldwide (e.g. wheat, rice, soybeans, and most trees), are adapted to moderate temperature and sunlight environment (Leegood, 1999). Plants using C_4 carbon fixation (or the Hatch-Slack pathway) represent a more advanced adaptation to more extreme environments, using spatial separation of initial CO_2 fixation and the Calvin cycle, occurring in the mesophyll cells and bundle sheath cells, respectively (Sage, 2004). The main characteristics of C_4 plants (e.g. maize, sorghum and sugarcane) is more efficient water use, making them better adapted to environments with higher temperatures, high light intensity and high aridity (Downes, 1969; Björkman, 1976; Sage, 2004). Some plants, such as cacti and pineapples, use the crassulacean acid metabolism (CAM photosynthesis), which is a further

adaptation for improved water use efficiency, and tend to be commonly found in arid environments (Winter *et al.,* 2005). The CAM plants minimize photorespiration by separating the Hatch-Slack pathway and the Calvin cycle temporally. In the daytime, CAM plants shut their stomata to reduce evapotranspiration, but still photosynthesize. During nighttime, the stomata are opened, allowing CO_2 to diffuse into their leaves and be fixed into oxaloacetate by Phosphoenolpyruvate carboxylase (PEPC; Osmond, 1978).

1.2.3.2 Plant lipid biomarkers

In prehistoric hunting and gathering societies, foraged wild plant foods often dominated the human diet and nutrition. With the collection techniques developed, various plant resources, seeds, fruits, leaves, nuts, etc., were extensively exploited by human ancestors (Zeder, 2012a). Except for foods, plant resources could also fulfil a variety of other uses, including medicine, clothing, technology, e.g. glues and binders, even containers, i.e. bamboo in South China utilized as a container in prehistory (Bar-Yosef *et al.,* 2012). Although plenty of wild plants can be eaten raw, processing raw plants prior to consumption was a crucial innovation in human evolution, which reduces energy lose and improves plant palatability (Carmody and Wrangham, 2009). Direct archaeobotanical evidence of prehistoric plants cooking (e.g. charred plant deposits, starches and phytoliths) are not readily recovered from archaeological sites, especially for the sites excavated in the 1900's in China where advanced recovery technologies were not routinely applied (e.g. phytolith, pollen analysis and soil flotation; Yanjiusuo, 2010). Pottery vessels, as a thermally resistant container, have been confirmed as being used to cook botanical resources in prehistory by ORA performed in Africa, Europe and the Americas, allowing an alternative means of investigating of plant use in antiquity (Evershed *et al*., 1991b; Eerken, 2001 & 2005; Cramp *et al.,* 2011; Dunne *et al.,* 2017). Considering the compositions of leaf waxes being isolated by immersed in organic solvents such as chloroform or hexane (Post-Beittenmiller, 1996), processing plant leaves by cooking or grinding releases epicuticular (leaf) waxes, the outer layers of higher plant surface, which are absorbed into the wall of pottery vessels (e.g. Evershed *et al.,* 1991b; Cramp *et al.,* 2011; Dunne *et al.,* 2017).

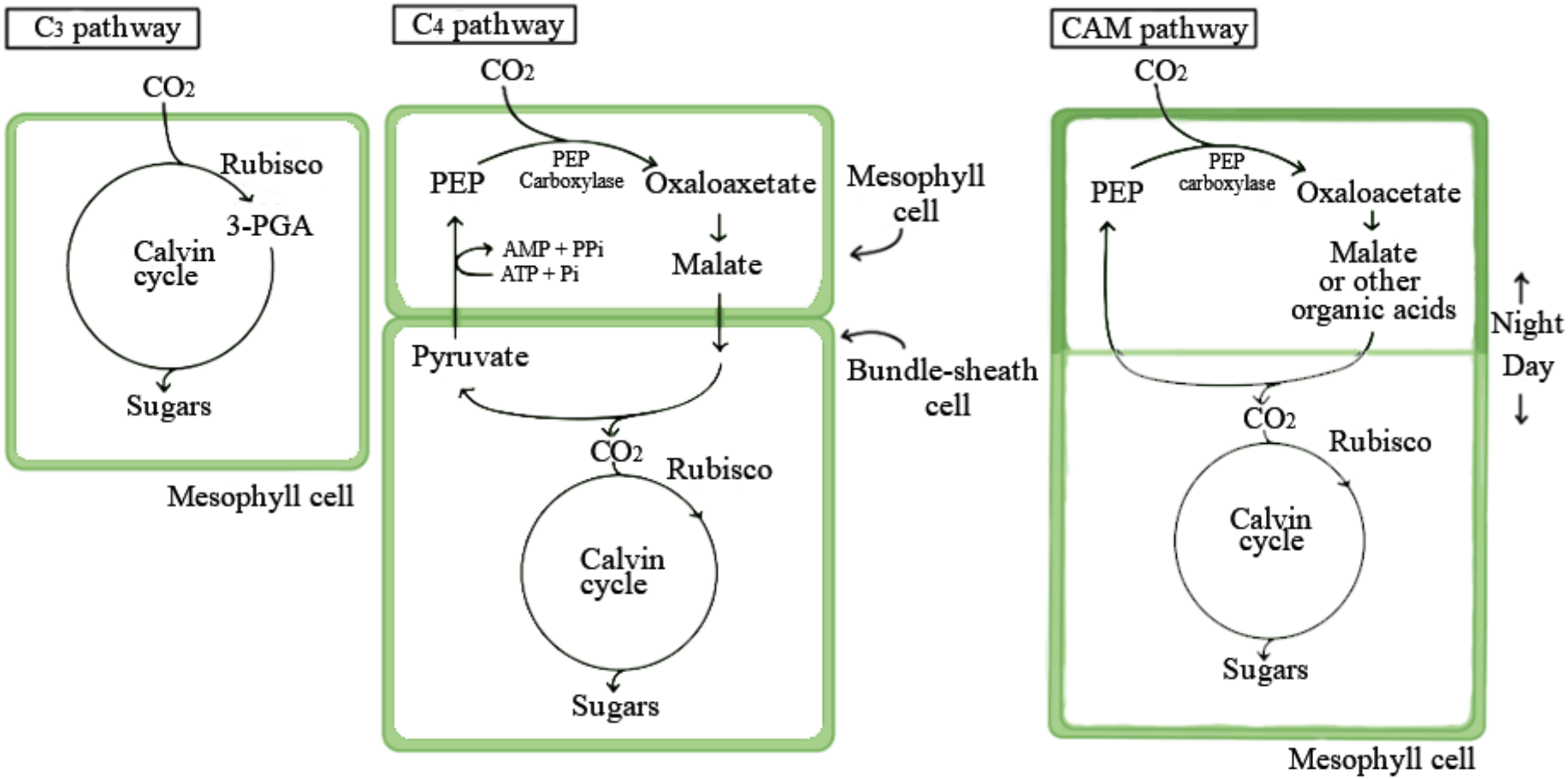


Figure 1-5 Diagrams illustrating the C_3, C_4 and CAM pathways (adapted from Khan Academy website).

Epicuticular waxes are complex mixtures of primarily *n*-alkanes, *n*-alcohols, even-numbered LCFAs and long-chain ketones (Eglinton and Hamilton, 1967; Tulloch, 1976). The proportions and predominance of each lipid class vary among and within species. One plant can display organ-to-organ or tissue-to-tissue differences, even growth stage differences, i.e. *n*-alkanes and ketones are the major components in *Brassica* leaf but are undetectable in maize and barley leaves (Post-Beittenmiller, 1996). Basically, long-chain *n*-alkanols are regarded as the major observed components of higher plant leaf waxes, displaying an even-over-odd carbon number predominance in the C_{25} to C_{35} range (Eglinton and Hamilton, 1967; Tulloch, 1976). Long-chain *n*-alcohols (primary) are assumed to be biosynthesised by fatty acid reductions, commonly displaying an odd-over-even carbon number predominance in the C_{22} to C_{34} range (Eglinton and Hamilton, 1967; Chibnall *et al.,* 1934), while secondary *n*-alcohols are principally present in C_{29} or C_{31} (Tulloch, 1976). Most waxes probably contain some monoester (Tulloch, 1976). Detailed compositions of leaf waxes are shown below (Table 1-1). These saturated lipids are stable and relatively resistant to degradation when absorbed and protected in the matrix of the fired clay wall (Evershed *et al.,* 1991b).

Table 1-1 Summary of plant lipid compositions (adapted from Chibnall *et al.,* 1934; Eglinton and Hamilton, 1967; Tulloch, 1976).

Compositions	Carbon numbers	Dominant carbon number
n-alkanes	C_{25}-C_{35}	Odd
n-alcohols	C_{22}-C_{34}	Even
Fatty acids	C_{16}-C_{34}	Even
Aldehydes	C_{21}-C_{35}	Even
Wax esters	C_{32}-C_{64}	Even

Additionally, ancient people have acquired more dependable supplies of food, exploiting greater varieties of food such as cereal or seeds. Early evidence of these plant-based food items such as charred grains or macrofossils can be scarce or inconclusive. Targeting their specific biomarkers, e.g. palm seeds is rich in unsaturated fatty acids (e.g. oleic acid) which survive across long timescales, protected by seed skin (Copley *et al.,* 2001), and cereals yield specific lipids, alkylresorcinols and plant sterols (Hammann and Cramp, 2018).

1.2.3.3 *n*-alkanes

In general, the presence of *n*-alkanes in archaeological pottery vessels is associated with the processing of epicuticular waxes from plants and, rarely, *n*-alkane distributions can be correlated to plant species (i.e. the profile dominated by C_{31} *n*-alkane is potentially indicative of C_3 or C_4 wild grasses or lake margin plant origins; Evershed *et al.,* 1991b; Dunne *et al.,*

2017). The chain length of *n*-alkanes normally ranges from 25 to 35 carbon atoms with odd-over-even distributions (Eglinton and Hamilton, 1967; Tulloch, 1976), present in lipid extractions as the biomarker of plants, however, they may be absent from lipid extracts due to poor solubilities in polar extraction solvents (Correa-Ascencio and Evershed, 2014), i.e. the lipid distribution extracted from the Takarkori or Uan Afuda potsherds processing plant resources (Dunne *et al.,* 2017).

Despite originating from plant waxes, *n*-alkanes are also present in fossil fuels (petroleum, bitumen, oil, etc.). The latter are readily distinguished from plant derived *n*-alkanes based on the carbon number distribution, as the alkanes fractions in fuels have similar abundances of even and odd carbon number homologues whereas plant *n*-alkanes display a distinctive odd-over-even carbon number predominance (Eglinton and Hamilton, 1963; Hofmann *et al.,* 1992). Modern plants including C_3, C_4, and CAM exhibit a significant predominance of odd-over-even carbon numbered *n*-alkanes (Collister *et al.,* 1994). Average chain length (ACL) and the carbon preference index (CPI) are used as a convenient means of numerically expressing carbon number distributions of aliphatic lipids, such as *n*-alkanes.

Within plants the ACL can potentially be used to characterize *n*-alkanes from different plant families and plant functional types (PFT) processed within pottery vessels. The calculated value converts *n*-alkane abundance to respective chain length numbers which has been found to vary substantially between different species and PFT, e.g. the Pinaceae averaging at *n*-C_{27}, the Cupressaceae averaging at *n*-C_{32} (Eglinton and Hamilton, 1967; Diefendorf *et al.,* 2011).

$$ACL = \frac{(25n\text{-}C_{25} + 27n\text{-}C_{27} + 29n\text{-}C_{29} + 31n\text{-}C_{31} + 33n\text{-}C_{33} + 35n\text{-}C_{35})}{(n\text{-}C_{25} + n\text{-}C_{27} + n\text{-}C_{29} + n\text{-}C_{31} + n\text{-}C_{33} + n\text{-}C_{35})}$$

Equation 1 Average chain length (ACL, *n*-Cxx represents the abundance of *n*-alkane Cxx; Eglinton and Hamilton, 1967)

CPI values are calculated using Equation 2 and have been found to vary between 1.6 to 82.1 and can be used broadly to assign the plant origin (Diefendorf *et al.,* 2011).

$$CPI = 0.5\left[\frac{(n\text{-}C_{25} + n\text{-}C_{27} + n\text{-}C_{29} + n\text{-}C_{31} + n\text{-}C_{33})}{(n\text{-}C_{26} + n\text{-}C_{28} + n\text{-}C_{30} + n\text{-}C_{32} + n\text{-}C_{34})} + \frac{(n\text{-}C_{25} + n\text{-}C_{27} + n\text{-}C_{29} + n\text{-}C_{31} + n\text{-}C_{33})}{(n\text{-}C_{24} + n\text{-}C_{26} + n\text{-}C_{28} + n\text{-}C_{30} + n\text{-}C_{32})}\right]$$

Equation 2 Carbon preference index (Bray and Evans, 1961).

1.2.4 $\delta^{13}C$ values of plant lipids

The carbon isotope composition of plants is determined by the different pathways of photosynthesis, namely: C_3, C_4 and CAM (Collister *et al*., 1994). Thus, the $\delta^{13}C$ values of plant lipid constituents extracted from potsherd residues can sometimes be used to identify the categories of plant commodities processed in pottery, to some extent, revealing the composition of the plant populations in ecosystems around settlements (Cramp, 2008; Dunne *et al.,* 2017). In C_3 plants, the atmospheric CO_2 is fixed by RuBisCo directly, while in C_4 plants, it is fixed *via* PEPC (O'Leary, 1981). Consequently, C_3 and C_4 plants exhibited different $\delta^{13}C$ values. The carbon isotope compositions of C_3 plants typically ranges from -34‰ to -22‰, while C_4 plants range from -19‰ to -6‰ (Smith and Epstein, 1971). CAM plants, able to switch between using either the C_3 or C_4 pathways, have intermeddle $\delta^{13}C$ values ranging from -20‰ to -10‰ (Deines, 1980). Moreover, plant species intermediate for C_3-C_4 photosynthesis (most of them occur in genera having also both C_3 and C_4 species, e.g. *Parthenium hysterophorus* and *Mollugo verticillate;* Kennedy and Laetsch, 1974; Moore *et al.,* 1987) will display a C_3-like value in most instances because only a small fraction of the total carbon fixed in the bundle sheath (Von Caemmerer, 1992).

In addition to the characteristic fractionations caused by photosynthetic pathways, the $\delta^{13}C$ values of vegetation can also vary in response to climate/environment or even with the different parts of plants (e.g. leaf wax lipids are depleted than the biomass; Heaton, 1999; Bi *et al.,* 2005). Any environmental factors causing a plant's photosynthetic efficiency to change will be reflected in the $\delta^{13}C$ values. If C_3 plants grow in hotter and drier environments, they will yield more enriched $\delta^{13}C$ values compared with the same C_3 plants growing under cooler and wetter conditions (Heaton, 1999).

1.2.5 Long-chain ketones

Long-chain ketones are known to be the constituents of higher plant leaf waxes (Tulloch, 1976). The abundance of long-chain ketones in archaeological pottery vessels is associated with the processing of leafy plants, coupled with other higher plant biomarkers (e.g. *n*-alcohols and *n*-alkanes), and normally present in a homologous series from *n*-C_{24} to *n*-C_{33}. In addition to a component of higher plants, ketones have also been detected after high-temperature heating (over 300 °C) of fatty acids absorbed into unglazed pottery matrix, displaying in $C_{31,}$ C_{33} and C_{35} homologues (Fig. 1-6; Evershed *et al.*, 1995; Raven *et al.*, 1997). Additionally, the origin

of ketones can also be determined isotopically, the $\delta^{13}C$ values of C_3 plant resources are more depleted than animal fats (Evershed *et al.*, 1995).

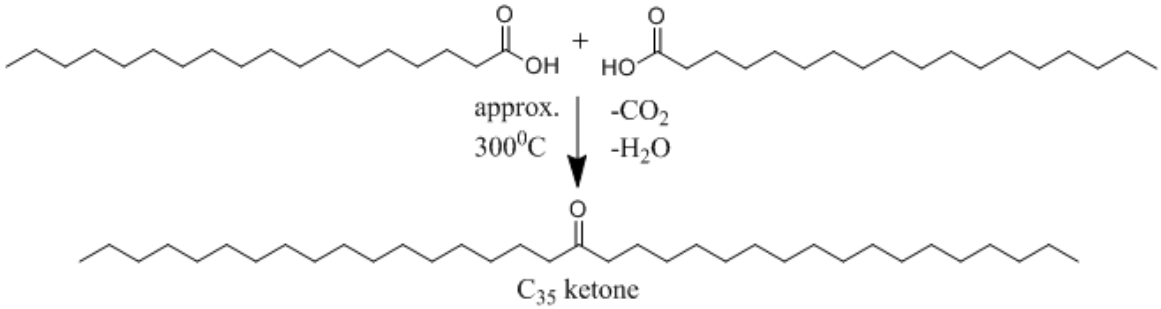


Figure 1-6 Formation of long-chain ketones by heating fatty acids (Evershed *et al*., 1995).

1.2.6 Terpenoids

Terpenoids, or terpenes, are natural polycyclic compounds, consisting of isoprene units linked together to form chains or cyclic structures (Pollard and Heron, 2008). Di- and triterpenoids are ubiquitously distributed across plants and animal kingdoms. Sometimes, triterpenoids are major constituents of waxes, for example, ursolic acid is found in the fruit and leaf waxes of apples (Fernandes *et al.,* 1964). The presence of terpenoids in antiquity can be used as indicators of plant exploitation (Hayek *et al.,* 1990). Di- and triterpenoids have been widely identified as constituents of plant resins but are rarely biosynthesised in the same tissue (Mills and White 1977; Evershed *et al*. 1997). Natural terpenoid resins would have served multiple technological functions in the past, e.g. as adhesives, waterproof, coating agents and glues, or as ingredients of flavorings, incense, cosmetics, medicines and even mummy balms (Robinson *et al.,* 1987; Buckley and Evershed, 2001; Buckley *et al.,* 2004; Regert, 2004; Colombini *et al.,* 2005; Mitkidou *et al.,* 2008; Pollard and Heron, 2008).

Coniferous resins, obtained from pine trees (the genus *Pinus,* part of the family Pinaceae) are typical diterpenoid products found widely in association with pottery vessels in temperate regions (Pollard and Heron, 2008). The characteristic diterpenoids are abietic, dehydroabietic, pimaric and isopimaric acids (Fig. 1-7). Such terpenoids are particularly susceptible to structural alteration, e.g. fresh *Pinus* resins comprises a high abundance of abietic acid, while dehydroabietic acid, the main product of oxidative dehydrogenation of abietic acid, is a highly characteristic biomarker of pine resin in aged samples (Pollard and Heron, 2008; Reber and Hart, 2008). Pine pitch, produced by heating pine resin is characterised by the presence of retene and methyl dehydroabitate (Mitkidou *et al*., 2008).

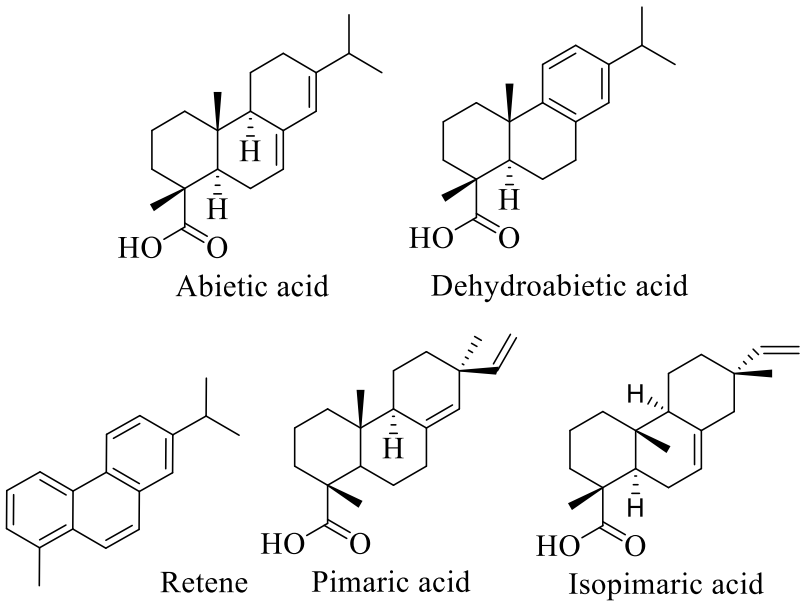
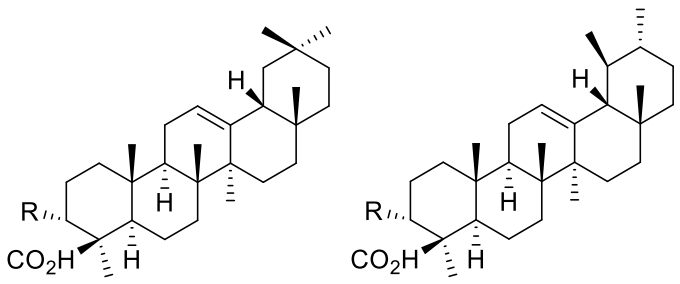


Figure 1-7 Structures of pine derived diterpenoid resin acids (Keeling and Bohlmann, 2006).

Frankincense (olibanum), which is the best known of aromatic gum-resin, is obtained from trees of various *Boswellia* spp. (family Burseraceae). It has been applied for thousand years as an incense material, also part of mortuary rite (Clarkson *et al.,* 2002; Brettell *et al.,* 2015). The most characteristic components are a group of pentacyclic triterpenoids acids, α-boswellic acid, 3-*O*-acetyl-α-boswellic acid, ß-boswellic acid, 3-*O*-acetyl-ß-boswellic acid, which obtained from recent or ancient frankincense (Fig. 1-8; Evershed *et al.,* 1997).



R =-OH: α-boswellic acid

R = -OAc: 3-*O*-acetyl-α-boswellic acid

R =-OH: β-boswellic acid

R = -OAc: 3-*O*-acetyl-β-boswellic acid

Figure 1-8 Main triterpenoids of frankincense (Mathe *et al*., 2004).

Birch bark tars, produced by the pyrolysis of bark from *Betula* spp., in excess of 350°C, have been recognized widely in antiquity as components of hafting, waterproofing or adhesive materials (Hayek *et al.,* 1990; Urem-Kotsou *et al.,* 2002; Lucquin *et al.,* 2007; Pollard and Heron, 2008). The animal fats mixed with birch bark tar and used as an adhesive have been investigated in several studies (Regert *et al.,* 1998; Dudd and Evershed, 1999). Triterpenoid compounds, namely betulin, lupenone and lupeol and their degradation products, have been identified as indicators of birch bark tar (Fig. 1-9; Pollard and Heron, 2008).

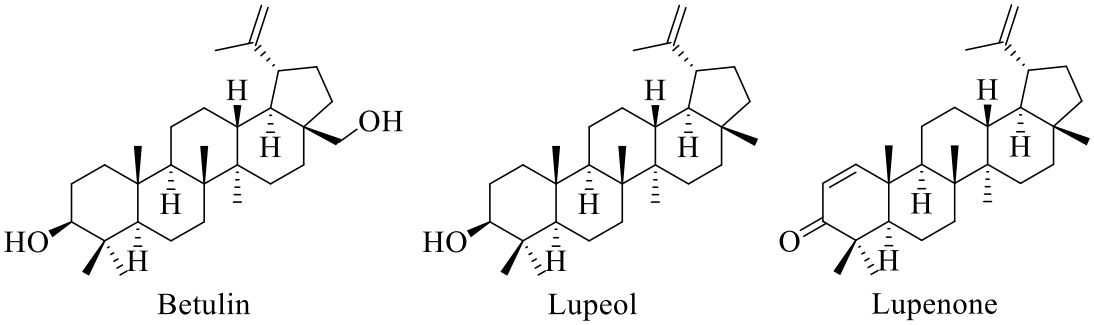


Figure 1-9 Chemical structures of birch bark tar derived triterpenoids (Dudd and Evershed, 1999).

1.2.7 The identification of aquatic commodities

Aquatic resources, such as fish, molluscs and marine mammals, provide a valuable source of nutrition to the human diet. Indirect artefactual finds (e.g. fishing hooks, spears or nets) of prehistoric human procuring aquatic resources have been excavated from ancient sites in Europe, Africa and Asia, albeit rarely (Marean *et al.,* 2007; Cortés-Sánchez *et al.,* 2011; Liu and Chen, 2012). With the exception of shells the skeletal remains of many aquatic organisms are fragile and small in size, making them poorly preserved over long archaeological timescales. Indeed, archaeological evidence for prehistoric exploitations of aquatic resources is much less readily obtained compared to terrestrial animal product consumptions (Cramp and Evershed, 2014). However, the processing of aquatic commodities within pottery vessels can be identified through a range of aquatic lipid biomarkers. There are three main types of aquatic biomarkers, ω-(*o*-alkylphenyl) alkanoic acids (APAAs), vicinal dihydroxy fatty acids (DHYAs) and isoprenoid fatty acids (Hansel *et al*., 2004; Evershed *et al*., 2008a; Hansel and Evershed, 2009).

One of these classes of aquatic lipid biomarker includes the isoprenoid fatty acids, 4,8,12-trimethyltridecanoic (4,8,12-TMTD), 3,7,11,15-tetramethylhexadecanoic (phytanic) and 2,6,10,14-tetramethylpentadecanoic (pristanic; Fig.1-10) acids (Hansel *et al.,* 2004). These multiple-branched fatty acids are formed *in vitro* from the metabolism of the phytol of chlorophyll. However, the rumen of terrestrial mammals and micro-organisms also convert phytol into phytanic and less abundant pristanic acids (Hansen, 1966; Mize *et al.,* 1966; Patton and Benson, 1966).

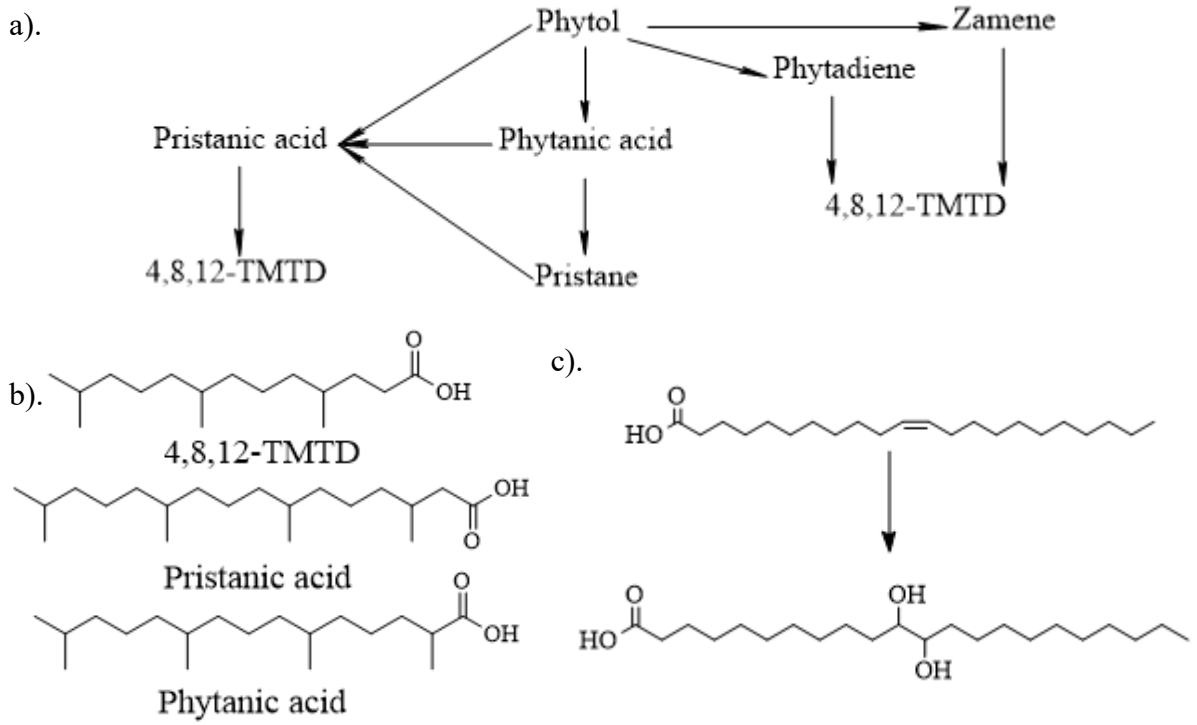


Figure 1-10. a) Scheme of degradation of isoprenoid fatty acids from phytol (Ackman and Hooper, 1968); b) chemical structures of isoprenoid fatty acids; c) scheme of DHYA formation (Hansel and Evershed, 2009).

Fresh marine fats/oils are characterised by mono- and polyunsaturated fatty acids (Ackman, 1964). They are unstable being prone to oxidative degradation during pottery use and burial (Evershed *et al.,* 2008a). DHYAs, ranging from C_{18}-C_{22}, are oxidative products of unsaturated fatty acids, and can be formed at room temperature. The position of the hydroxyl group reflects the original double-bond position of the unsaturated fatty acid precursors (Fig. 1-10; Hansel and Evershed, 2009; Hansel *et al.,* 2011).

APAAs are not natural-occurring compounds, most readily formed through heating of triunsaturated fatty acids ($C_{16:3}$, $C_{18:3}$, $C_{20:3}$ and $C_{22:3}$) at temperatures over 270°C within the ceramic matrix. APAAs are only formed when the metal ions are present in the pottery clay, as the clay acts as a catalyst (Fig.1-11). Once formed, they are stable and survive over long archaeological timescales. Laboratory experiment have shown that triunsaturated fatty acids, all of which are abundant in marine fats and oils, can give rise C_{16}-C_{22} APAAs, thereby providing additional evidence of processing aquatic products (Hansel *et al.,* 2004; Evershed *et al.,* 2008a).

Among these aquatic lipids, the C_{18} DHYA, C_{18} APAAs, phytanic and pristanic acids are possibly to be found in terrestrial origins, i.e. vegetable oils and fats also contain high concentrations of the $C_{18:1}$ which is the precursor of C_{18} DHYA (Ackman and Hooper, 1968;

Rossell, 1991; Evershed *et al.,* 2008a; Lucquin *et al.,* 2016a; Casanova, 2018). A suite of dihydroxy fatty acids, APAAs (of carbon number greater than C_{20}), isoprenoids and stable carbon isotope signatures of individual fatty acids can reinforce the interpretation of processing aquatic commodities in pottery vessels (Hansel *et al.,* 2004; Hansel and Evershed, 2009; Hansel *et al.,* 2011; Cramp and Evershed, 2014).

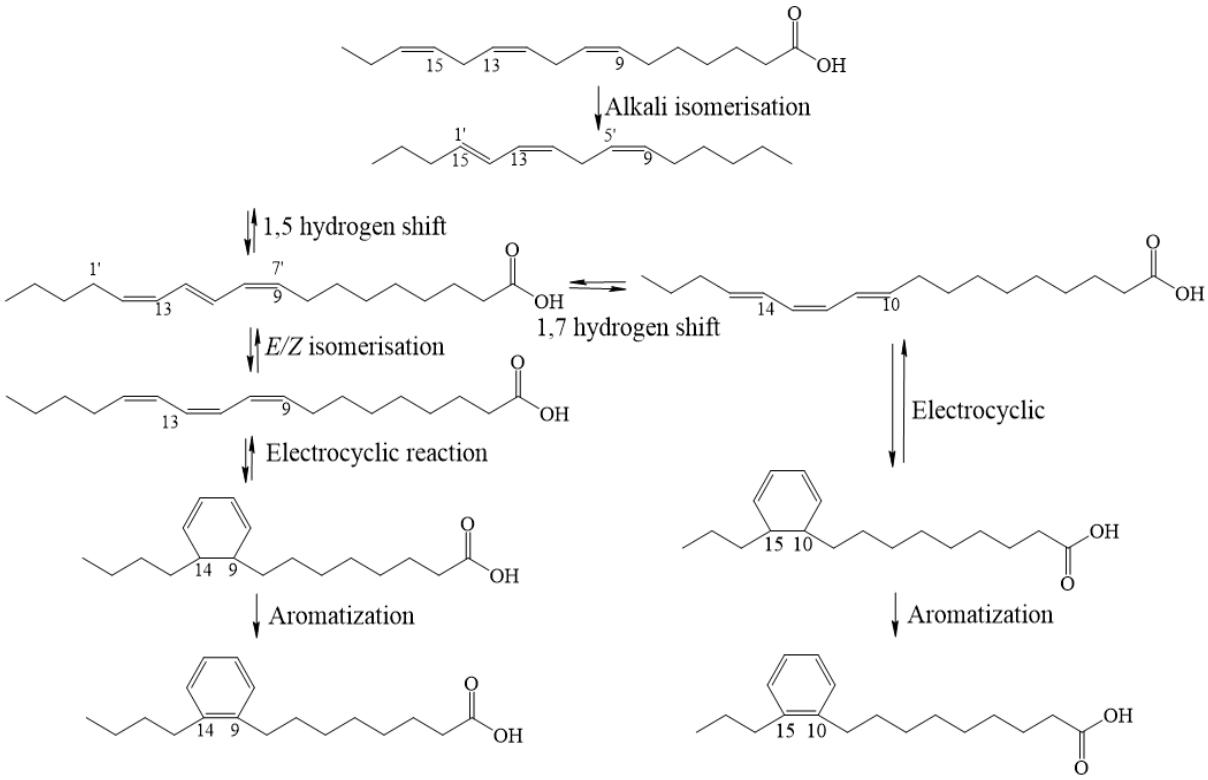


Figure 1-11 Formation of APAAs (adapted from Hansel *et al.,* 2004).

1.2.8 Beeswax

Beeswax of the honeybees (*Apis mellifera*, *Apis cerana*, *Apis dorsata*, *Apis laboriosa*, *Apis florea* and *Apis andreniformis*) has likely been a valuable and sough substance for human groups over long archaeological timescales, in China, honey was consumed by the 9,000 B.P., and honey hunting or beekeeping were common during the imperial period (Pattinson, 2018). The western honeybee became isolated from Asia species as desert developed in the Middle East and evolved into *Apis mellifera* which is the only species in Europe and Africa now. It has been evidenced widely present in ancient Egyptian bee iconography (Crane, 1999). Asia has diversity of *Apis* species, and *Apis cerana* (also named Chinese honeybee) is a honeybee species indigenous to China, also the only one to be commercially exploited in beekeeping practices (Yang, 2001; Pattinson, 2018).

ORA has been applied to trace the use of honeybee through prehistory (Heron *et al.,* 1994; Evershed *et al.,* 1997c; Roffet-Salque *et al.,* 2015), mainly identified in the mixtures of fatty acids, *n*-alkanes, *n*-alkenes, and wax esters (Table 1-2; Tulloch, 1980; Aichholz and Lorbeer, 1999; Regert *et al.,* 2001; Garnier *et al.,* 2002). The overall lipid composition of beeswax (*Apis* spp.) varies between species (e.g. *Apis mellifera*, *Apis cerana*, and *Apis florea)* while the content of individual compound class is not so distinctive (Aichholz and Lorbeer, 1999). The major fraction of beeswax is wax esters dominated by even-numbered monoesters, comprising alkyl palmitates, range from C_{38} to C_{52}, with C_{46} and C_{48} being the major components (Tulloch, 1971). Hydroxy-monoesters are highly diagnostic of beeswax and range from C_{40}-C_{54} with long-chain *n*-alcohols, esterified with 15-hydroxypalmitic acid (Aichholz and Lorbeer, 1999). More typical feature of individual honeybee species is the characteristic ratio of individual homologous compound, e.g. *Apis mellifera* yields higher abundance of LCFAs than *Apis cerana* (Aichholz and Lorbeer, 1999).

Aging of beeswax results in its compositions changing (Garnier *et al.,* 2002), notably, the hydrolysis of wax esters resulting in increasing the abundance of free *n*-alcohols, which are largely absent from fresh beeswax (Buckley and Evershed, 2001; Regert *et al.,* 2001; Evershed *et al.,* 2003; Roffet-Salque *et al.,* 2015).

Table 1-2 Fresh beeswax compositions (adapted from Aichholz and Lorbeer, 1999; Regert *et al.,* 2001; Garnier *et al*., 2002).

Compositions	Carbon numbers	Dominant carbon number
Saturated fatty acids	C_{20}- C_{36} (even-over-odd)	C_{24}
n-alkanes	C_{23}-C_{31} (odd-over-even)	C_{27}
n-alkenes	C_{27}-C_{35} (odd-over-even)	-
Monoesters	C_{38}-C_{52}	-
Hydroxymonoesters	C_{40}-C_{54}	-
n-alcohols	C_{33}, C_{35}	-

1.3 Applications of organic residue analysis in Chinese archaeological ceramic vessels

Organic residue analysis of pottery from archaeological sites has been applied across Europe, Egypt, East Asia, the Near East and Africa, providing valuable direct archaeological evidence for ancient lifestyles and approach for detecting the uses of archaeological pottery vessels residues (Copley *et al.,* 2001; Evershed *et al.,* 2008b; Craig *et al.,* 2013; Roffet-Salque *et al.,* 2015; Dunne *et al.,* 2017). However, as an empirical archaeological approach, organic residue

analysis has rarely been carried out in Chinese archaeological studies, with only a few cases utilizing this method as support in their projects. Their study designs, results and conclusions were rather variable.

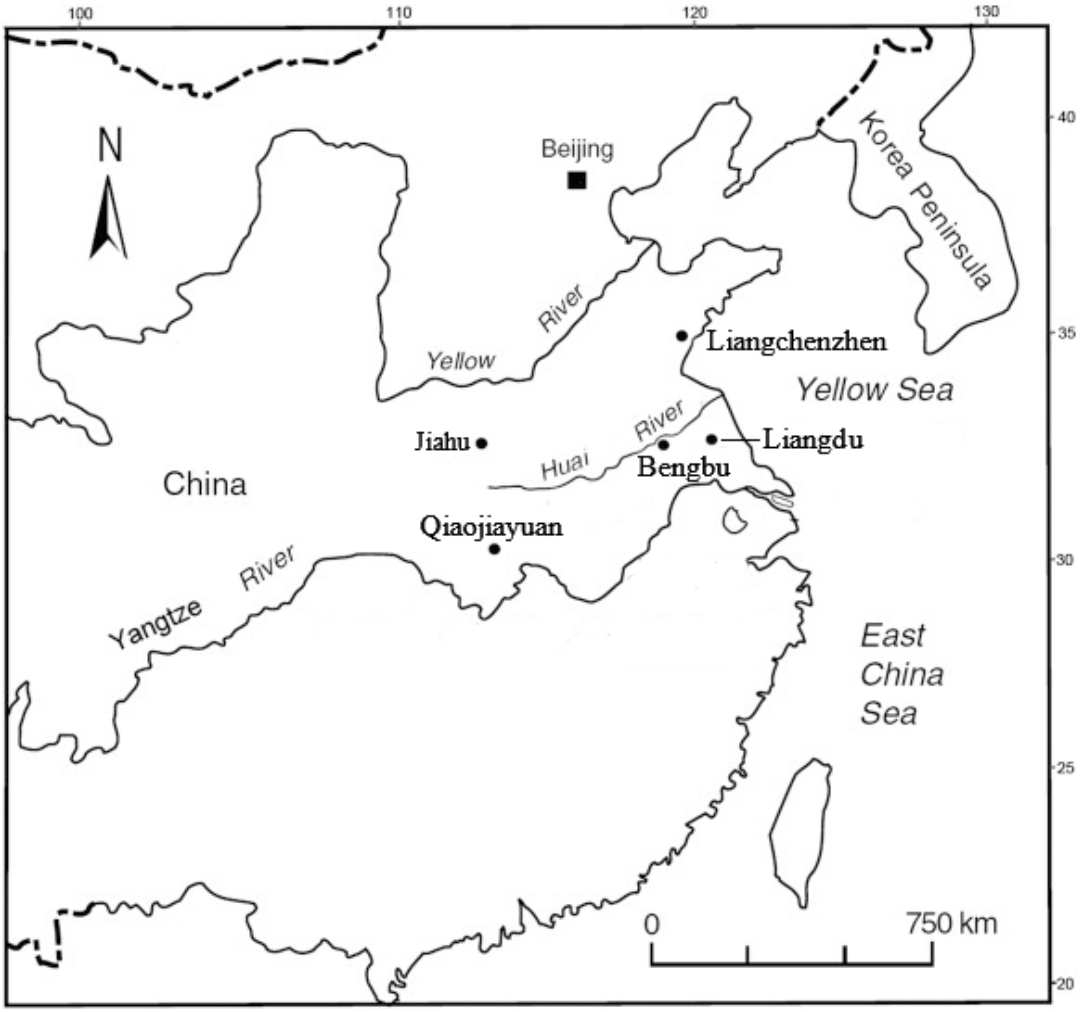
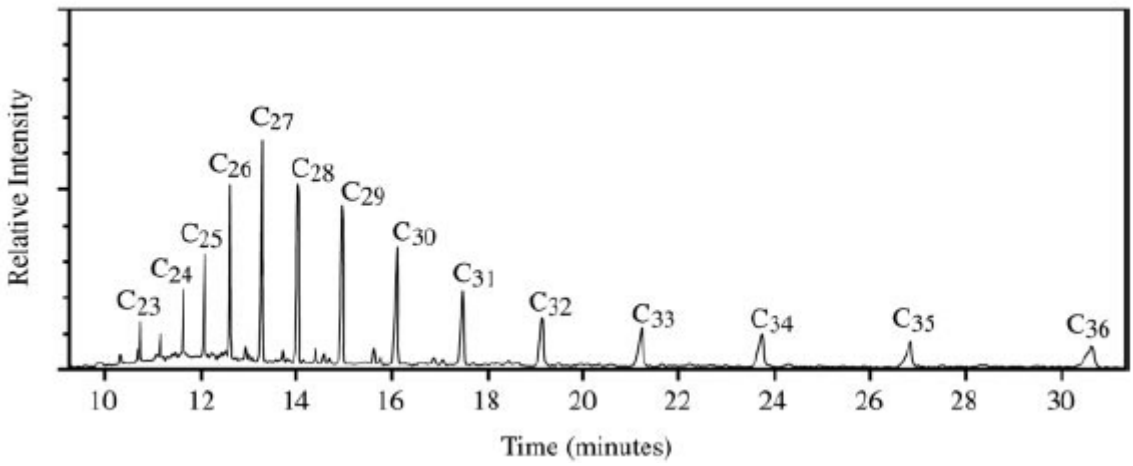


Figure 1-12 Distribution of prehistoric cultures in China mentioned in this chapter.

As a fairly advanced Chinese Neolithic settlement, Jiahu, Henan, occupied around 9000 to 7700 B.P. (Li and Chen, 2012). The emergence of early pig domestication at the site from at least 8600 B.P. has been confirmed, also with foxtail millet and rice cultivations (Cucchi *et al.,* 2011; Liu and Chen, 2012). McGovern *et al*. (2014) applied lipid biomarker chemical analysis to pottery jars (direct methanol extraction), concluding the presence of hybrid beverages produced by fermentation of C_3 plants, rice and grapes. They hypothesized honey, *Chrysanthemum* and tree resins such as China fir were supposed to have been added to the alcoholic beverages as specific aromatic additions (McGovern *et al*., 2014). However, from the gas chromatogram shown, displaying a profile comprising *n*-alkanes, these appear to be petroleum-derived (Fig. 1-13). The petroleum-derived *n*-alkanes as the distribution displays

little or no dominance of odd over even chain lengths, exhibiting a CPI value close to 1 (Free and Pancost, 2014). If they were of plant wax or beeswax origin, the abundance of odd-numbered *n*-alkanes is usually much higher than even-numbered *n*-alkanes. Although the distribution of plant or beeswax-derived *n*-alkane is possible to present in the petroleum-contaminated samples (Volkman *et al.,* 1992), the authors did not state how they determined the origin of the *n*-alkanes (e.g. CPI or solvent extraction for detecting wax esters; Aichholz and Lobeer, 1999; Free and Pancost, 2014).

a).



b).

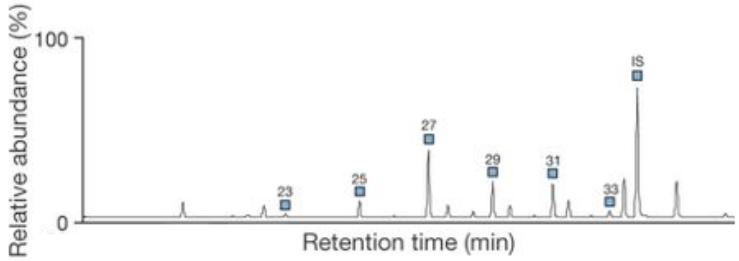


Figure 1-13 Comparison of three partial GC profiles a) the chloroform/methanol extract from Jiahu (McGovern *et al*., 2014), *n*-alkanes are given by C_X. The distributions of homologous *n*-alkanes are without significant odd-over-even carbon numbers, representing the origin of alkanes is petroleum (Volkman *et al.,* 1992); b) Partial HTGC profile of the lipid extract of an archaeological potsherd containing beeswax. Square, *n*-alkanes; IS internal standard. Lipid profile showed significant odd-over-even *n*-alkane distributions (Roffer-Salque *et al.,* 2015).

In another study two turquoise-inlaid bronze swords were excavated at M_4 tombs in Qiaojiayuan, Yun county, Hubei Province, exhibited some sticky whitish pastes (Fig. 1-14). The tomb culture dated back to around 6th to 5th century B.C. Luo *et al.* (2011) applied FTIR (Fourier Transform Infrared Spectroscopy) and XRD (X-Ray Diffraction) to the pastes, trying to reveal whether binding agents were involved in the inlay technique in early China. These exposed pastes were used to stick the turquoise into the sword. The authors concluded the components were almost pure beeswax (Luo *et al.,* 2011), although a better approach would have been to GC and GC/MS as these methods would have produced unequivocal identifications of beeswax.

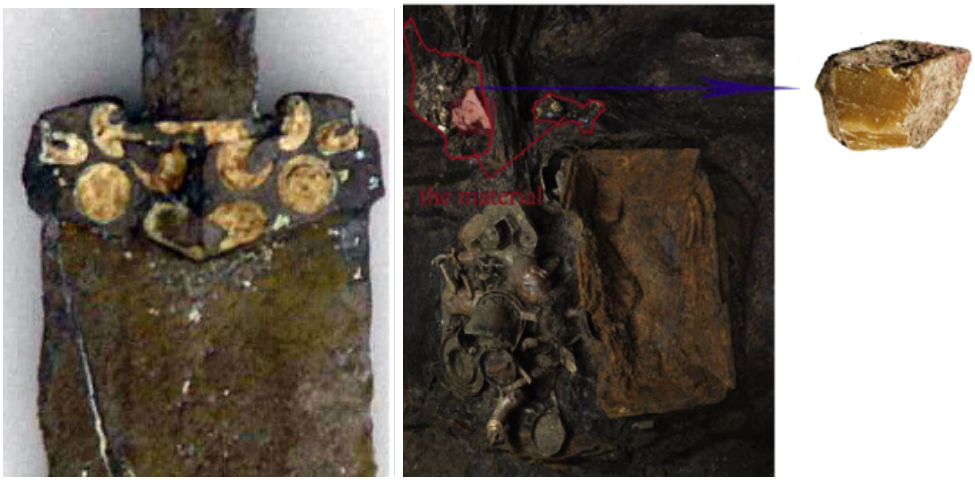


Figure 1-14 From left to right. The whitish pastes were exposed after the inlaid turquoises dropped of the sword excavated from Qiaojiayuan (Luo *et al.,* 2011); the material recovered from two adjacent lumps at the royal cemetery in Jiangsu Province (Ma *et al.,* 2015).

Two amorphous lumps of an unknown material were excavated from a large royal cemetery, dating back to 1st century B.C., located in Jiangsu Province. The archaeological material showed a translucent, amber-colored, soft core (Fig. 1-14). By conducting FTIR, GC and GC-MS on both the outer surface and the inner part, GC-MS showed the material was composed of saturated hydrocarbons, fatty acids, alcohols and esters which are known components of beeswax, and are consistent with the FTIR spectrum, which was similar to that of fresh beeswax (Ma *et al.,* 2015).

Most relevant to this thesis Lanehart (2015) discriminated the extracted lipid residues from pottery collected from Liangchengzhen, a Longshan (*ca.* 4600-3900 B.P.) site located in Shandong Province, by the *n*-alkane fractions. Accelerated Solvent Extraction (ASE) was applied to 64 potsherds from each stratigraphic phase, and GC-MS used to quantify the ratio of C_{15}: C_{17} and C_{25}: C_{27} *n*-alkanes and then basing lipid amount ratios to distinguish marine or terrestrial food origin, also with the carbon isotope values of C_{15} and C_{17} *n*-alkanes. However, C_{25} and C_{27} *n*-alkanes were in low abundance (poor signal-to-noise ratio), thus, as lipid components, author only focused on study *n*-C_{15} and *n*-C_{17} compared to those of the marine and terrestrial reference samples. As *n*-alkanes often only occur in low abundance in the lipid extracts, or even do not survive during aging (Dunne *et al.,* 2017), it was surprising the study did not focus on fatty acids as these are acknowledged as the most reliable biomarkers for animal product processing in pottery vessels. Based on the results the authors claimed a wide range of marine foodstuffs had been processed in the vessels, along with the ubiquitous distribution of millet and plants. For their thesis, the author only utilized alkane peak

abundances to determine the lipid origin (Lanehart, 2015) which is an unproven method for determining the origin of lipids in this context in archaeology.

An interesting study by Yang *et al.* (2014) performed proteomics on a amorphous specimen collected from a mummy of Early Bronze Age cemetery of Xiaohe (3980-3450 B.P.), Xinjiang Province, aiming to discriminate and quantify protein compositions. Authors identified ten out of a total twelve ancient samples as kefir cheese due to their common compositional hallmarks consistent with a self-made kefir curd (Yang *et al.,* 2014). These samples could also have been further investigated by ORA to provide fatty acid and stable isotope evidence the existence of cheese, but this was not performed.

Finally, Zhou applied solvent extraction to several painted potsherds from Tomb Shuangdun-1 of the Spring-and-Autumn period in Bengbu in her master project, trying to recover any absorbed herbal plant lipids from sherds. The results presented in her thesis were inconclusive, with no gas chromatograms presented (Zhou, 2011).

1.4 Prior identification of food processing among hunter-gatherer societies

The transition from fishing, hunting, and gathering to cultivation societies was one of the most profound evolutions in human history (Childe, 1936; Thomas, 1999; Bocquet-Appel, 2011). Reconstruct culinary practices of preagricultural societies pave the way for further understand the origin of agriculture and domestication. By the hunter-gatherer societies, organic residue analysis has shown ceramic material is extensively used to process food. Investigation into dietary practices and the exploitation of natural products, commonly by a range of diagnostic criteria and proxies, have been covered through European to Asian hunter-gatherer societies (Eerkens, 2001; Dunne *et al.,* 2012, 2017; Lucquin *et al.,* 2016b; Shoda *et al.,* 2017). The high abundance of both animal and plant products in pottery vessels in Africa before food production has been observed at Takarkori and Uan Afuda. This has been found as the earliest processing plant within ceramic vessels so far (Dunne *et al.,* 2017).

Eerkens (2001) investigated 74 potsherds selected from late prehistoric hunter-gatherers in Northern America, applying solvent extraction and screened under GC and GC-MS, to infer that plants represented the overwhelming majority of foods cooked within Western Great Basin pottery. Only 30% of vessels showed evidence for having been used to cook animal products, with 18 sherds suggesting he pots were used to process mixtures of plant and animal products.

ORA was performed 101 potsherds from Incipient Jōmon vessels from 13 sites Japan, displaying indications of vessels use due to the presence charred surface deposits on revealed the predominance of degraded aquatic oils, freshwater and/or marine (Craig *et al.,* 2013). A further study of 143 potsherds from the Jōmon site of Torihama in western Japan, intermittently occupied from the Late Pleistocene to the mid-Holocene, over 50% produced an interpretable residue containing aquatic biomarkers, confirming the exploitation of marine resources, coupled with terrestrial species (Lucquin *et al.,* 2016b).

For early Holocene of Korea, a total of 55 potsherds and 12 foodcrust from two coastal locations of Sejuk and Jukbyeon-ri site were analysed using direct methanol extraction. Of the potsherds containing interpretable amounts of lipids, diagnostic compounds for aquatic resources were observed on 41% of potsherds and 47% of the foodcrust from Sejuk, and 22% of potsherd and 89% of the foodcrusts from Jukbyeon-ri. The GC-C-IRMS results suggested he vessels were used mainly o process aquatic resources (Shoda *et al.,* 2017).

2. The archaeological context

2.1 Review of Chinese prehistoric subsistence

The original accepted traits of the Neolithic period, the so-called 'Neolithic Revolution' are defined as sedentism, plant cultivation, animal domestication and pottery production (Childe, 1936). However, this archaeological model is no longer fits with expressing the Neolithic in region such as in China where early pottery was first made and used 10 millennia or more before the emergence of agriculture (Wu *et al.,* 2012). A more adopted definition of 'Neolithic Revolution' is the shift from forager to producer societies (Bocquet-Appel, 2011), and also, to constitute a Chinese style archaeology, where indigenous characteristics should be considered (Liu, 2005). The current proposed date of the Neolithic period in China is approximately from 12,000 B.P to 4,000 B.P (Liu and Chen, 2012).

Table 2-1 The chronology of the archaeological periods in China (Yanjiusuo, 2010).

Archaeological phases	Date (B.P.)
Mesolithic	not known
Initial Neolithic	11,000-9,000
Early Neolithic	9,000-7,000
Middle Neolithic	7,000-5,000
Late Neolithic	5,000-4,000

Given China's wide ranging geomorphic and climatic variability, settlement types during the Neolithic of China varied considerably likely influenced by local environmental conditions (Liu, 2005; Liu and Chen, 2012). Thus, archaeologists divide the whole country geographically into seven ecological zones, based on their environmental characteristics, agricultural potential and current provincial units. (Fig. 2-1; Chang, 1994). Throughout the long prehistoric timescale, the climatic and geomorphic changes have in many ways affected economic adaptations and social organizations. Both natural and human forces have dramatically altered China's landscape, and the human response to the ever-changing environment has manifested diverse cultural traditions (Liu and Chen, 2012; Yanjiusuo, 2010).

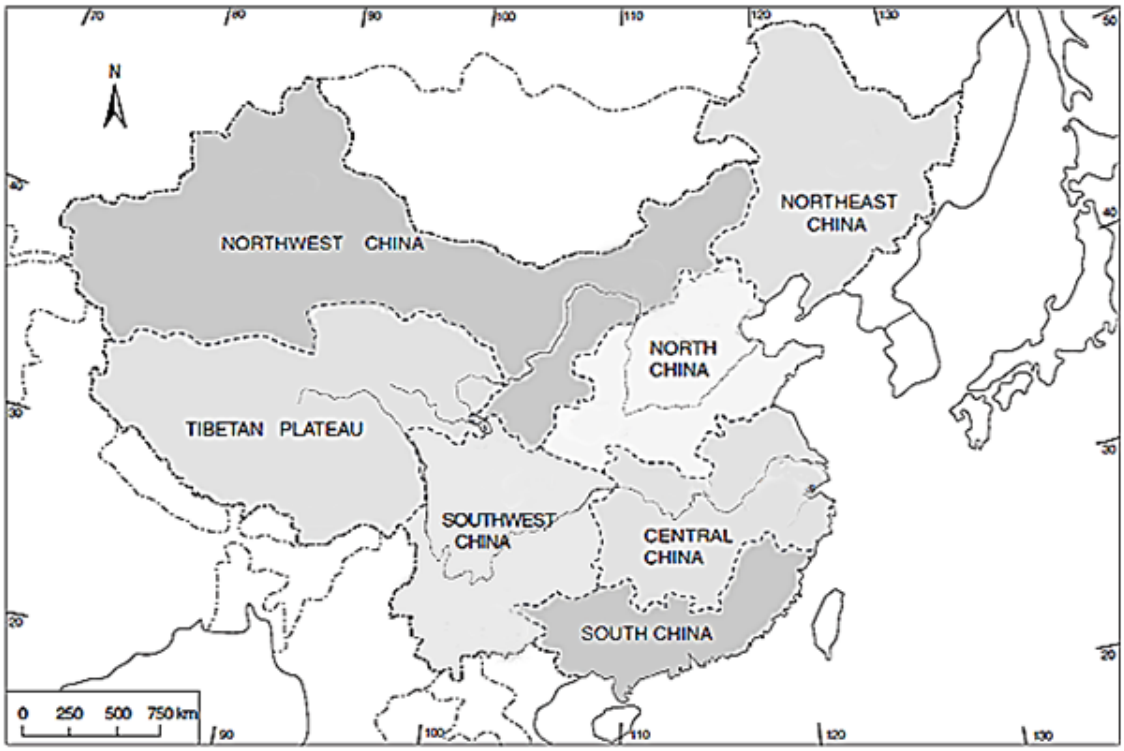


Figure 2-1 geographical divisions of China (adapted from Liu and Chen, 2012).

The start of the initial Neolithic period in China is characterized by a gradual climatic change from cool and dry to warm and wet conditions during the Pleistocene-Holocene climate transition, which provided more food resources (ca. 11,000-9,000 B.P.). The major human adaptions included an increase in sedentary lifestyles, the emergency of pottery, intensified exploitation of wild plant resources, and possibly the initial stage of agriculture. Human were initially tuber-, seed- and nut-collectors, thus, the technological improvements implied the utilization of an expanded range of foods, achieved by cooking them in ceramics and the availability of storable foods, particularly nuts and cereals, which made the sedentary lifestyle possible (Liu, 2005; Baker, 2006; Liu and Chen, 2012). As foragers, people constantly moved their residential bases to the food-rich locations, as exemplified in the site clusters found in North China, i.e. Shizitan (21,000-8,500 cal. B.P.; Guojia, 2004). In contrast, collecting communities formed logistical organizations to procure foods, even far from their residential bases, stored relatively long-lasting foods, and gradually developed sedentary villages. This situation is best manifested in Shangshan, but also observable, to a lesser extent, in Donghulin (11,000-9,000 cal. B.P.; Zhao, 2006), and Nanzhuangtou in Hebei (16,300-14,700 cal. B.P.; Baoding City Institute for Administration of Cultural Relics, 1992). It must be pointed out that not all regions in China show a trend of changing subsistence strategy during this period. Some groups in remote areas, like the arid/semiarid areas of north China, the higher altitude regions

of Northwest, or some cave sites in south China, apparently continued Epipaleolithic traditions well into the Holocene, continuously relying on hunting, fishing and gathering. The lag of subsistence-settlement changes in those regions seems to suggest that the temperate ecosystem, with seasonal natural resources, may have been a necessary environmental condition for encouraging technological and social development during this initial period (Yanjiusuo, 2010; Liu and Chen, 2012).

Early Neolithic cultures in China appeared throughout 9,000-7,000 B.P., people employed a broad-spectrum subsistence strategy which included foraging for wild resources and early cultivation. The high proportions of grinding stones among toolkits in the Liao, Yellow, and lower Yangzi Rivers during the early Neolithic period suggest intensive exploitation of wild plants over broad geographical regions. Significant development of food production is indicated by the presence of domesticated cereals at many sites in the Liao, Yellow, and Yangzi River regions and harvest methods such as sickles or harvest knives (Harlan, 1992; Liu, 2005; Liu and Chen, 2012), i.e. the Cishan culture in Hebei (Sun *et al.,* 1981). Not until the middle Neolithic period did agriculture become intensified in many regions of China. Sedentism seemed to be initially established in most regions, indicated by cultivation traces (Liu, 2005). Domesticated animals included pigs, dogs, and possibly chicken (Zhou, 1984; Cucchi *et al.,* 2011), as plant cultivation mainly included millet and rice (Denham *et al.,* 2018). The emergence of sedentism also accelerated the development of technologies for resource management and food storage, which contributed to social and economic innovations and supported more complex societies emerging in the later period. Current knowledge of regional differences in material culture, such as ceramic forms, tool types, and settlement patterns, are closely related to the variety of food resources in different areas and correspondingly diverse food acquisition strategies adopted by local populations (Yanjiusuo, 2010; Liu and Chen, 2012).

Two developmental trends corresponded to the Middle Neolithic period (9,000-7,000 B.P), which coincided with the Mid-Holocene Climatic Optimum (a warmer and more humid phase; Liu, 2005). First, each region's Neolithic culture became complex. Favorable climatic conditions were the environmental backdrop for settlements increasing dramatically in number and size (i.e. Shaanxi, Miaodigou sites doubled in number compared to the previous Banpo phase) and underly the flourishing of fully developed Neolithic cultures into the whole country (Liu, 2005; Liu and Chen, 2012). Cultivation became a primary and ubiquitous food procurement mode in both North and South China, leading to a steady growth in population

and indicating a transformation from mobile hunter-gatherer or semi-sedentism to a cultivational-based Neolithic economy (Liu and Chen, 2012). Second, most regional cultures became more extensively distributed, and interregional communications among them became intensified. Neolithic farming exerted pressured on over-exploited agricultural areas and subsequently spread to more extensive and remote arable geographic lands, resulting in ever broader distribution of Neolithic assemblages in China (Yanjiusuo, 2010; Liu and Chen, 2012). As consequence population dispersion was gradually observed in most regions of China, i.e. about 800 Yangshao sites have been discovered in Henan and about 2,000 sites in Shanxi, even extending northward to Inner Mongolia (Yang, 1991; Zhang *et al.,* 1999; Liu and Chen, 2012). Such interactions between each interregional culture (called 'the Longshanoid Horizon') was linked by migrants from one community to another, probably conducted by boat *via* water channels (Yanjiusuo, 2010; Liu and Chen, 2012).

Cultural development during the late Neolithic period (7,000-4,000 B.P) was diverse (Liu and Chen, 2012). As the East Asian monsoon began to weaken, in the Asian monsoon region, the temperature became colder, and the amount of monsoonal broad-leaved trees reduced (Liu, 2005). The highest population densities observed in the main river areas motivated the agricultural production to be overexploited, and caused deterioration of the local ecosystem, forcing occupants to adjust their socioeconomic strategies. People living in central alluvial plains probably moved to the north plateau areas. Simultaneously, across the landscape among the main river regions, the decline in site numbers and abandonment of regional centres was accompanied by the human density of each community reaching its peak (Liu, 2005; Liu and Chen, 2012). From a cultural evolutionary perspective, the Late Neolithic was the transitional period from the Neolithic complex societies to the first state-level societies. Even some advanced late Neolithic cultures show traits that later became essential characteristics of civilizations, i.e., bronze metallurgy. In contrast, hunter-gatherers still continuously dominated the landscapes in other areas, such as Xinjiang, potentially keeping employing low-level food production (Yanjiusuo, 2010; Liu and Chen, 2012).

2.2 Review of Chinese early pottery production

Various hypothesises have been proposed concerning the origins of pottery, such as early building construction, a durable cooking container or as a human adaption to environment transition during the initial Neolithic period. Pottery in China appearing earlier than 9000 ^{14}C B.P. is regarded as 'early pottery'. Early pottery sites are distributed in the northern and

central/southern regions. Current knowledge about the earliest known pottery vessels in the world are those excavated from Xianrendong cave, Jiangxi Province. The earliest pottery found was radiocarbon dated up to ~20, 000 B.P. (Fig. 2-2; Wu *et al.,* 2012), leading to a new understanding that the time of the first pottery produced in China, predating by millennia to sedentary plant cultivation (Cohen, 2013). Pots represented a fundamental techno-economic innovation in Neolithic period, with the emergence of pottery providing equipment to cook foods before consumption, contributing increased food choice and enhanced nutrition (Carmody and Wrangham, 2009; Liu and Chen, 2012). Pottery is a symbol of reduced residential mobility, increased requirement from continuous subsistence system or intensive exploitation of natural resources (Liu and Chen, 2012). While in some regions, like the Near East, American Southwest and eastern Mediterranean Pakistan, pottery appeared after the beginning of plant cultivation, and food preparation connected to grinding stones or making porridges and beer (Fuller and Rowlands, 2011).

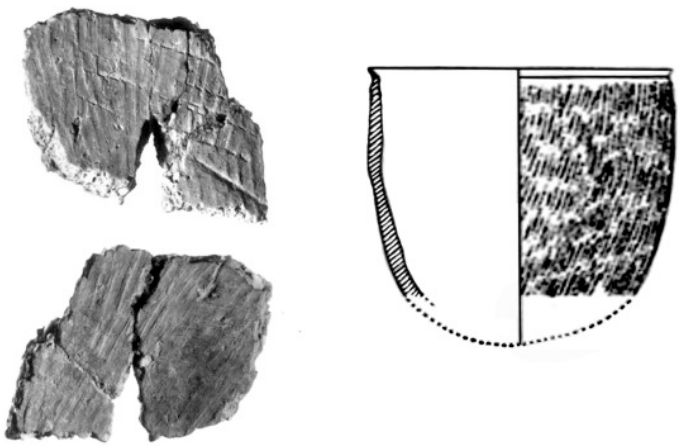


Figure 2-2 Early pottery from Xianrendong cave. Left: cord-marked potsherds dating back to ~20,000-19,000 B.P. Right: A reconstruction of the possible shape of an early pottery vessel from Xianrendong (adapted from Cohen *et al.,* 2017).

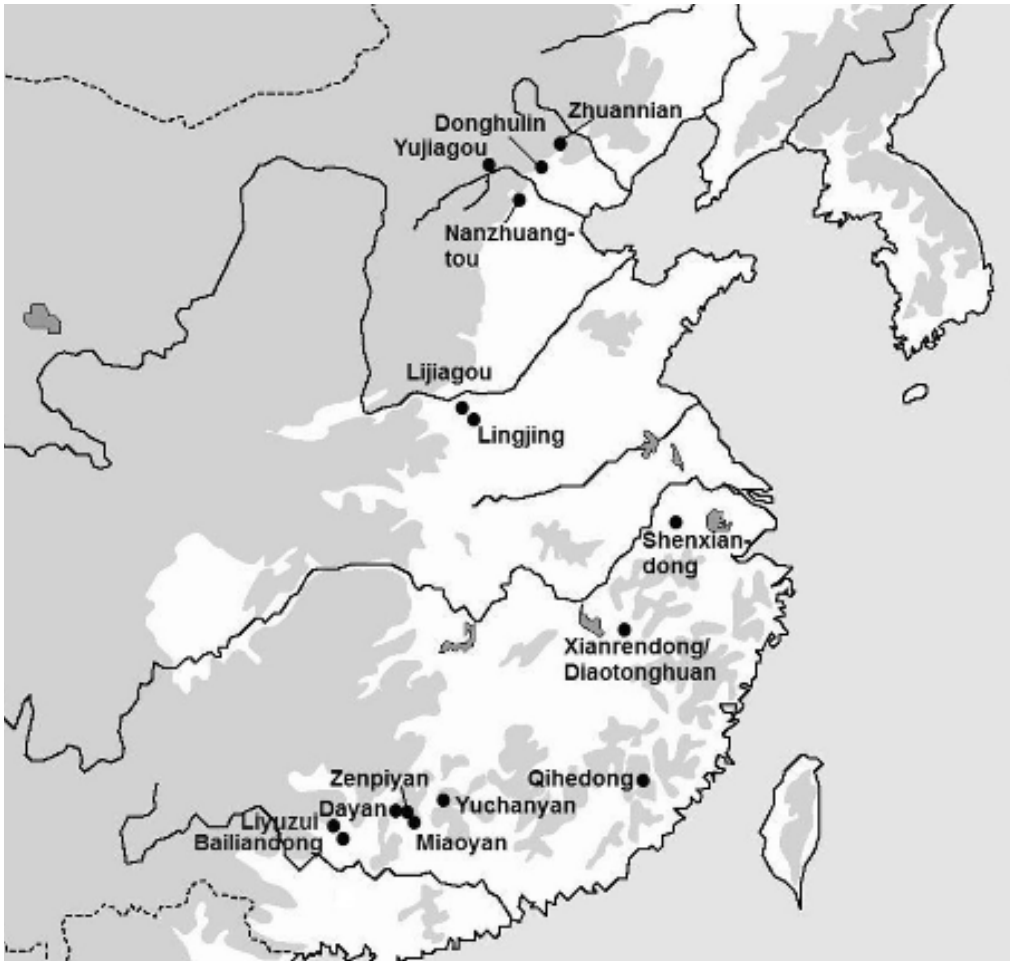
Regional variations existed among the early ceramic industry, regarding types of the vessels, materials used in the paste, and vessel function. Initial characteristics of early pottery unearthed from Neolithic sites suggest low firing temperatures, thick walls, and simple forms. The features of early pottery in central and Southern China is quite similar (hereafter referred to as central/southern China). Archaeological comparisons between the early pottery of northern and central/southern China show two different archaeological cultural traditions: one named the "cave-dwelling culture", mainly distributed in the south, while the other was the so-called "microlithic culture" in north China (Fig.2-3; Zhang, 2002; Liu and Chen, 2012; Li *et al.,* 2017). In terms of Northern China, laminar and microblade industries eventually dominated the Late

Pleistocene archaeological record (Brantingham *et al.,* 2004). Archaeological finds of early pottery are quite rare among the northern nomadic peoples. Only six Neolithic sites are known as yielding early pottery, mainly comprising deep or shallow vessels, both with thick walls and flat bases. Most excavated potsherds are undecorated, a few are finished with cord-mark and rolling patterns. They seem to be utilized for cooking with direct heating agent as charcoal remains have been found on the surface. Combined with the archaeological data, these early pottery in Northern China may be related to microblade technologies, as these two technologies appear as coupled discoveries at the majority sites (Liu and Chen, 2012; Li *et al.,* 2017).

The emergence of pottery in central/southern China predates that of Northern China. Early pottery found in central/southern China, represented by cave sites (e.g., Yuchanyan, Xianrendong, Zengpiyan and Dayan), occupied mainly by hunter-gatherer societies (Chinese Academy of Social Science *et al.,* 2003; Cohen *et al.,* 2017). Occupants of these sites exploited a complex collector strategy to maximize their capabilities for food procurement. The reconstructed pottery vessels show a pointed basic form of deep pot with thick wall and round base (U-shaped; Fig. 2-3) decorated with tapped cordage marks. In contrast to Northern China, microblade industries or grinding stones never accompany early pottery in central/southern China. Instead, core and flake and cobble implements were present throughout the early Holocene (Bar-Yosef *et al.,* 2011). Based on shell midden deposits observed and geographic location of these cave sites, early pottery is associated with processing freshwater shellfish (Lu, 2010; Liu and Chen, 2012).

During the early Holocene period, pottery became more widespread, as Neolithic people gradually reduced their mobility and increased their reliance on food production, forming an important component of adaption strategies. The development pottery industries began to appear diversely in Neolithic communities to fulfil specific requirements, e.g. rituals, feasting or cooking; Yanjiusuo, 2010; Liu and Chen, 2012). Potters likely intentionally designed pottery forms and selected certain types of materials, inclusions or pastes to determine the vessel functions and improve performance during use, i.e., the pottery types, *guan* and *ding*, were assumed to have been used for cooking meat, while *yan* was believed to have been used for processing vegetables or grains (Underhills, 2002; Liu and Chen, 2012).

a).



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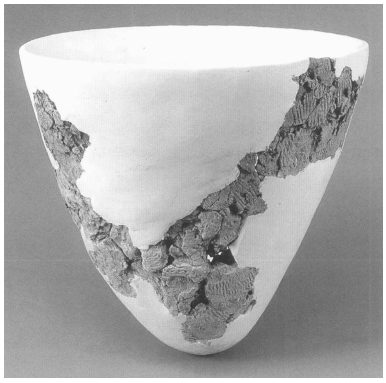

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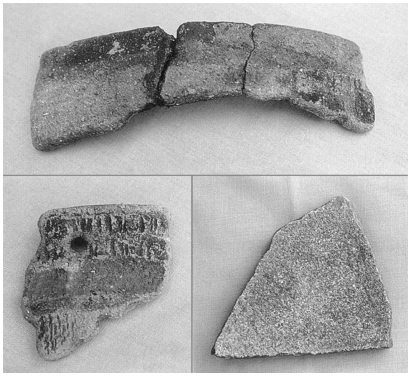


d).

Figure 2-3 a) Location map of early pottery sites in China; b) Pottery from Yuchanyan; c) pottery from Nanzhuangtou; d) pottery bowl with a ring-shaped loop discovered at Shangshan; (adpated from Zhang, 2002; Liu and Chen, 2012; Li *et al.,* 2017).

2.3 Zengpiyan

The early Neolithic of South China began with a group of cave sites, dating between 7,500 to 6,000 B.C: Xianrendong and Diaotonghuan in Jiangxi; Zengpiyan, Baozitou in Guangxi; Qingtang in Guangdong. No direct evidence of agricultural production has been recovered from these sites, but it has been claimed that cultivation began in this period, based on relatively advanced levels of cultivation evident at slightly later sites (Pearson and Underhill, 1987; Liu and Chen, 2012).

As the main study site in this thesis, Zengpiyan is an early Neolithic cave in South China. It is located in a limestone plain on the southwestern fringes of the Dushan Mountain in Guilin, Guangxi province. Based on stratigraphic sequences and artefacts excavated thus far, cultural deposits at the Zengpiyan can be divided into five phases, with the first phase dated to 12,000-11,000 cal B.P. and the last to 8,000-7,000 cal B.P. (Table 2-2). Since its first excavation in the 1970s, the site has attracted much academic attention because the pig bones excavated have been identified as possibly from domesticated pigs and the site was believed to be the earliest archaeological site with rice farming by some scholars. However, the stratigraphy and cultural chronology were not clear, and no plant remains have been ever reported from this excavation. To resolve these problems, Zengpiyan was re-excavated by the Institute of Archaeology in 2001 with multidisciplinary approach (Fig. 2-4; Chinese Academy of Social Science *et al.,* 2003).

Table 2-2 The chronology of Zengpiyan culture (Chinese Academy of Social Science *et al.,* 2003).

Archaeological phase	Date (cal B.P)
Phase I	12,000-11,000
Phase II	11,000-10,000
Phase III	10,000-9,000
Phase IV	9,000-8,000
Phase V	8,000-7,000

The site consists of a sequence with the main entrance facing southwest. This natural cave was utilized as a dwelling place, whether it was only a seasonal habitat is not yet confirmed. The prehistoric deposits are piled highest near the entrance, sloping left at about 10°. An overall cultural coverage of roughly 260 m^2 was excavated, revealing 21 pits,18 burials, 921 potsherds and 63 stone tools. The artefact assemblage includes substantial amounts of lithic, bone, antler, and mollusc shell implements. Chronologies for Zengpiyan cultural phases are determined on

the calibrated radiocarbon dates obtained from deposits. The ^{14}C radiocarbon dating of shells unearthed from the site is back to 11310±180 B.P., the ^{14}C dating of animal bones is back to 7580±410 B.P. and nine thermoluminescence dates on Zengpiyan pottery ranged from 6,990 to 10,340 B.P. (Chinese Academy of Social Science *et al.,* 2003).

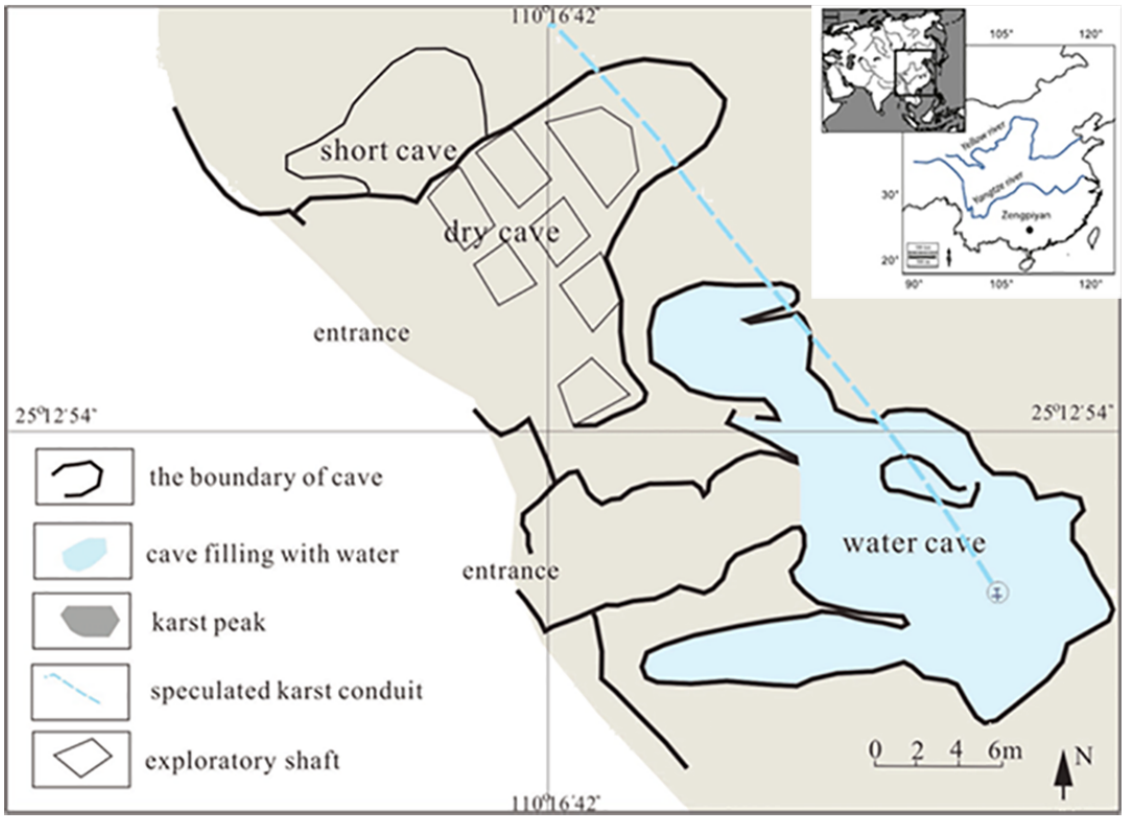


Figure 2-4 Schematic of the Zengpiyan cave, modified from Guo *et al.,* (2015) and Gucchi *et al.,* (2010).

2.3.1 Subsistence patterns

Faunal and floral remains at settlement sites can offer great insight into the modes of food procurement by past peoples. The fauna in a region is related to local climatic and

environmental conditions. Zengpiyan is located in southwest China, experiencing a relatively tropical/subtropical warm-moist climate with the temperature at Zengpiyan during the period of occupation higher than present-day (Fig.2-5). Variations in subsistence occurring among different cultural phases are likely to occur due to variations in ecological conditions or changing environmental conditions (Chinese Academy of Social Science *et al.,* 2003).

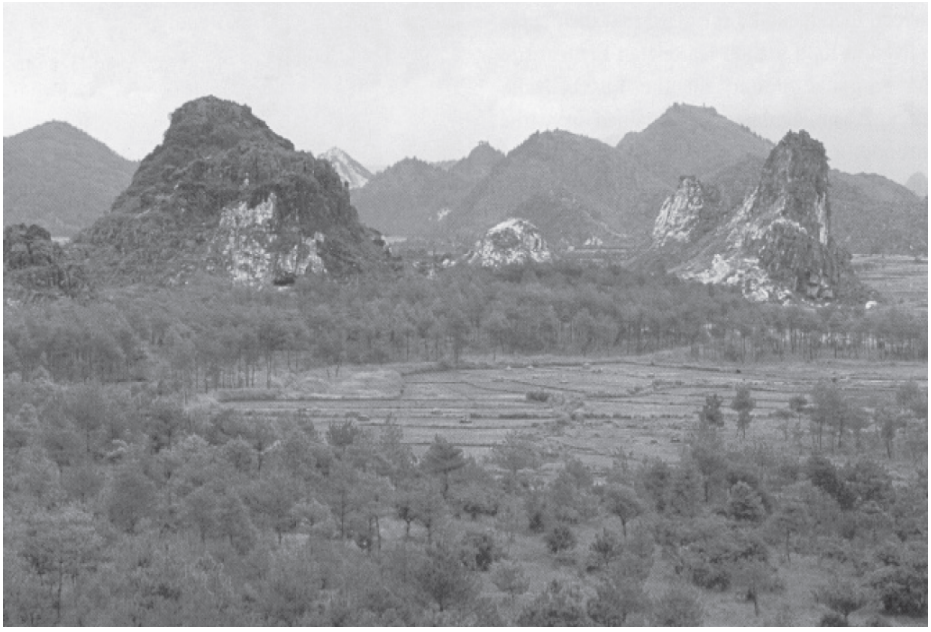


Figure 2-5 Photograph of Zengpiyan region taken in 1975 (Chinese Academy of Social Science *et al.,* 2003).

Subsistence activities and mobility patterns at Zengpiyan are not fully understood yet. From the ecofactual remains investigated so far, hunting and gathering appeared to be the major subsistence strategies for food procurement. Surrounded by small hills and the adjacent -Li river and inland streams, provided the site inhabitants ideal foraging conditions. There were no signs of early plant cultivation in the area, probably as a consequence of the site situated on the top of a craggy outcrop on the edge of Li River, only one meter above the river level. The immediate vicinity around the site was also probably unsuitable for agriculture (Chinese Academy of Social Science *et al.,* 2003; Guo *et al.,* 2015).

2.3.1.1 Faunal evidence

Substantial amounts of faunal skeletal remains unearthed in both excavations suggest the importance of hunting. Freshwater shellfish (dominated by Chinese mystery snails, *Cipangopaludina cahayensis*) and wild deer bones dominated in the total faunal assemblage, possibly representing the staple dietary foods. The identified amount of each animal taxon fluctuated between cultural phases, with shellfish exploitation in Phase II dropping

significantly (Fig. 2-6). Of the mammal assemblages can certainly be identified as belonging to each species thus far deer (mainly indigenous species *sika* and *muntjac*) was always in high abundance, at more than 80%, followed by pigs, although in relatively low numbers (less than 7%), with other mammal species (e.g. wild sheep or cattle) were present in very low abundance (Table 2-2; Chinese Academy of Social Science *et al.,* 2003).

Table 2-3 Composition of the identified faunal assemblages at Zengpiyan. Specimen excavated in 1973 were not counted in this table as the stratigraphy and cultural chronology are not clear and well-established (adapted from Chinese Academy of Social Science *et al.,* 2003).

Species		Phase I	Phase II	Phase III	Phase IV	Phase V
Shellfish		2752	560	23333	9643	29241
Vertebrates	Mammal	6012	2844	7590	3652	3600
	Fish	185	233	1162	423	324
	Bird	36	50	338	184	107
	Reptile	43	69	160	89	110

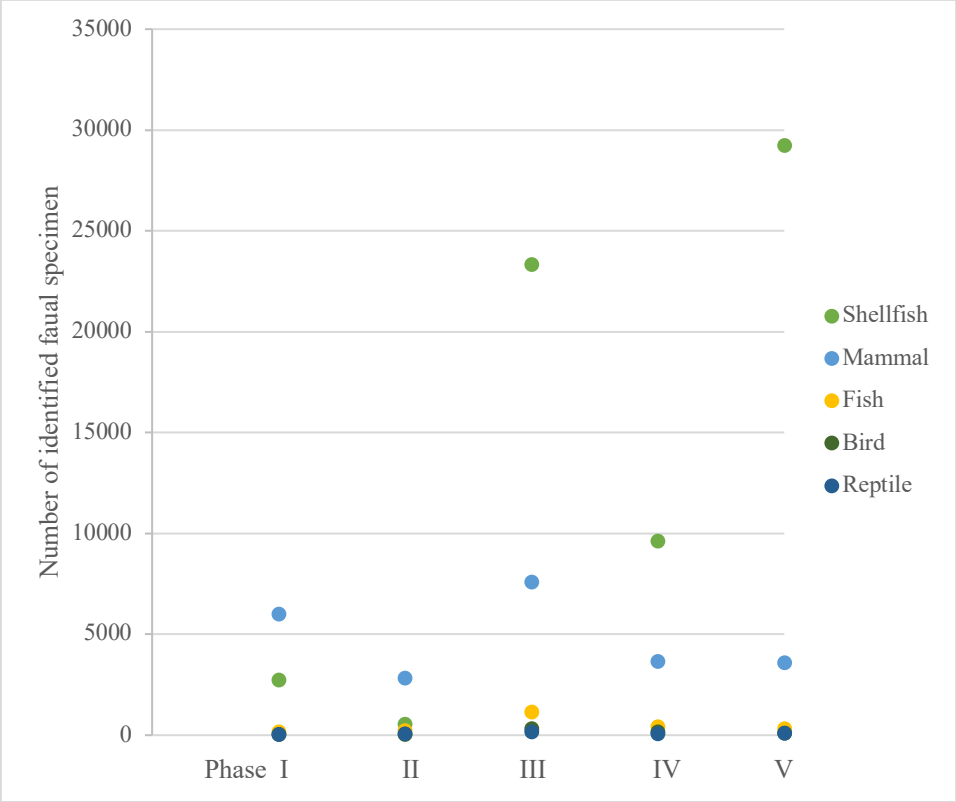


Figure 2-6 Graph showing fluctuations with phase of identified faunal remains excavated from Zengpiyan (Chinese Academy of Social Science *et al.,* 2003).

While sheep, goats and cattle were the main domesticated animals in Neolithic Europe and the Middle East, pigs are important domesticates for prehistoric human societies of East Asia, and

likely to be the earliest domesticates in many areas of China (Yuan, 2006; Jing and Flad, 2002). The timing of pig domestication in archaeological sites has engaged scholars for several decades. The hypothesis of pig husbandry in the Zengpiyan culture is based on the excavated tooth or jaw fragments found in the 1970s. The number of pig skeletons counted in this site is 67, of which about 40 of them can be assumed of accurate age. Estimate specific age relies on the timing of tooth eruption, which refers to the period of deciduous or permanent tooth growth to exposure through the gums. Another factor is tooth abrasion, normally, severer abrasion suggests an older animal. From the above, individual age at death of Zengpiyan pigs is as follows: eight (20%) were under one-year-old, 26 (65%) between the ages of one and two years, and the remainder (6; 15%) were older than 2 years. None of the excavated teeth are severely abraded. Such an age distribution is, if not caused by a special cause of nature, also unlikely to be a result of human hunting. The most likely explanation is that this distribution was the result of human domestication and slaughter. However, except for the age structure, there is no other solid evidence for the timing of the domestication of pigs in Zengpiyan (Payne, 1973; Chinese Academy of Social Science *et al.,* 2003).

The essential criteria zooarchaeologists rely on to determine pig domestication in archaeological sites are as follows:

1. Domestication is a strategic shift in human behaviour, causing an unintended innovation on distinctive morphological consequences (phenotype; Zeder, 2012b). The differing morphologies between wild and domesticated pigs, especially at the early stage of domestication, are subtle and not always easy to distinguish. Generally, the size of domesticated pigs' skull is smaller than wild ones. Also, the morphological changes occur in the teeth. Therefore, the signal of domestication can be shown through metric analyses of bone and teeth (Chinese Academy of Social Science *et al.,* 2003; Jing, 2008; Zeder, 2012b).
2. The age profile of animals unearthed at an archaeological site reflects animal husbandry. The age at which domestic animals are slaughtered by humans depends on the relative value placed on the different products that can be obtained from them (milk, meat etc), as different taxa ages are need for different purposes. Given the main product pigs provide is meat, the mortality age of domesticated pigs between 1-2 years old will be the most efficient herding strategy, as the optimum time point of meat-gain (Payne,

1973). The age at death of wild pigs excavated from archaeological sites is random rather than regular (Jing, 2008).

3. The ratio of females should be higher than males (Smith, 1995, Jing, 2008).

As mentioned above, the deduced age profile of the excavated pig remains is the only evidence supporting pig husbandry. The size of excavated pig teeth in Zengpiyan suggests the majority of pigs had reached the maximum size of the domesticated animals. The sex profile was not clear. Given all this evidence, it has been concluded that pig domestication was a low or non-existent contribution to the human subsistence practices during Zengpiyan period; at most the evidence probably shows preadaptation to domestication. (Smith, 1995; Chinese Academy of Social Science *et al.,* 2003).

2.3.1.2 Archaeobotanical evidence

No archaeobotanical remains were recovered in the trial excavation in 1973. Thus, the re-excavation in 2001 was subjected to archaeobotanic methods, including soil flotation, pollen and phytolith analysis and use-wear residue analysis. Abundant carbonised plant remains of roots and tubers were recovered and 82 well-preserved seeds belonging to more than 16 plant species were found during soil flotation. Phytolith analysis was only carried out in soil samples collected from each cultural layer in DT4 and BT3 pits. The retrieved phytoliths from cultural deposits were in low abundance, especially among the samples unearthed from the original excavation, Poaceae sector phytolith is the only identified phytolith type, no *Oryza* genus were observed, such as rice leaf types. Given both the result of phytolith analysis and flotation results, it is highly unlikely that Zengpiyan people practised rice agriculture. From Phase I to Phase V, both total identified phytoliths and carbonised plant remain numbers gradually increased. Starch residue analysis of stone or bone implements from each cultural phase showed each phase yielded only one specimen containing a relative high abundance of taro starch in the blade section. Given the abundance of taro starch in natural soil was found to be quite low, the presence on the blade section was correlated with tool function (Chinese Academy of Social Science *et al.,* 2003; Zhao, 2011).

Quantitative analysis of flotation, starch, and use-wear from botanical deposits at Zengpiyan indicates that the floral assemblages are dominated by tubers. *Pteridophyta* was the major local species and rhizomes were the main edible plants. However, it should be noted that a very low amount of plant remains were recovered overall. The low amount would be incorporated with the food selection. The plant species identified at the site would not have required processing

before consumption or short-term preserved. It has been suggested that the inhabitants potentially ate plants at collecting points instead of bringing them back to the cave (Chinese Academy of Social Science *et al.,* 2003).

2.3.2 Pottery technology of Zengpiyan

The prehistoric people of Early Neolithic period in South China largely relied on hunter-gatherer economies, with little evidence for cultivation. Pottery manufacture in this region predated grain-growing agriculture and even wild rice gathering was not the major economic activity of the people at this period (Chinese Academy of Social Science *et al.,* 2003; Denham *et al.,* 2018). Therefore, the appearance of pottery vessels was not related to grass seed gathering or consumption in this area and correlated within major human lifeway changes at the end of Palaeolithic (Chinese Academy of Social Science *et al.,* 2003).

Nine thermoluminescence dates on Zengpiyan pottery ranged from 6,990 to 10,340 B.P. (Wang and Zhou, 1983). They are sand red and greyish pottery, with muddy terra-cotta being the major type. All the pottery is hand-made, the wall is thick with low firing temperatures used (maximum temperature is less than 850°C). The ceramic clay is crisp and the form is simple, mainly including kettle, ca, sputum and few three-legged devices, with decorations mainly of 'rope', 'scratch' and 'basket' patterns. The details of the evolution of Zengpiyan pottery manufacture are discussed below.

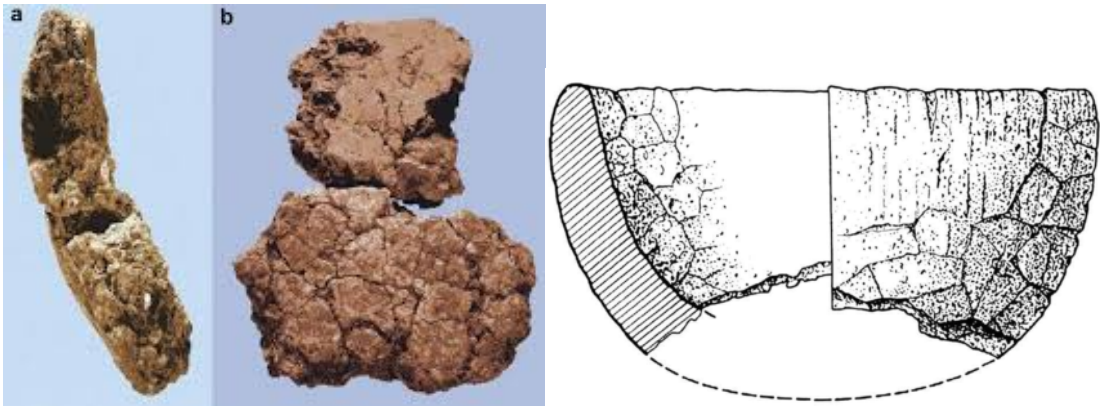


Figure 2-7 a) and b). Earliest potsherds excavated from Phase I, Zengpiyan; c). Reconstructed pottery vessel from potsherds unearthed from Phase I (adapted from Chinese Academy of Social Science *et al.,* 2003).

Cultural Phase I was characterized as associated with the initial appearance of pottery. The firing temperature was very low (probably ≤ 250°C). The pots were thick walled (2.9 to 3.6 cm) formed by hand-pinching and the fabrics very crumbly, containing un-sieved crushed

quartz (as large as 1.5 cm) as a tempering agent. The cracked outer surface is finished by rolling patterns, which might have been remains of the 'wiping off 'or 'smoothing off' efforts rather than intended decoration. Only one shape of pottery is seen, i.e. the large-mouth and shallow hemispherical form ('*fu*' in Chinese; Fig 2-7). Previous studies indicated that it is necessary to cook shellfish before consumption, otherwise the meat is not released unless the entire shell is crushed which would results in the meat also being crushed and contaminated with shell fragments making consumption unpleasant. Therefore, it is highly likely in Guangxi, the pottery industry was stimulated by freshwater shellfish consumption (Chinese Academy of Social Science *et al.,* 2003; Lu, 2010).

In the second cultural phase, the quantity of excavated potsherds increased although these were still fired at low temperatures. Slab building became the new prevalent manufacturing technique, and crushed calcite became the new preferred tempering agent, supplemented by crushed quartz. The walls became relatively thinner and the vessels taller, indicating improved techniques of body construction (Fig. 2-8). The concept of decoration appeared during this cultural phase, as a cord pattern instead of the way to smooth the surface; further, liner incisions and high relief occurred as decorations. More vessels types appeared, but, the majority were still mainly in '*fu*' shape (Chinese Academy of Social Science *et al.,* 2003; Lu, 2010).

The pottery of Phase III remained fired at a low temperature and crumbly with coarse calcite grains as the temper agent. Slab building remained as the major construction treatment. The potsherd matrix of this period had a laminated structure consisting of many thin layers, which showed a differentiation from the structure formed by slab building and might have been the consequence of technical immature during the production. Different sizes of cord marks are seen on the surface, but incisions and thumb-pressing decorations also occurred. A new style of round-based pot with a short neck and big mouth appeared (Fig. 2-8; Chinese Academy of Social Science *et al.,* 2003).

Both the quantity and shape diversity of pottery increased in Phase IV. Pottery vessels were assumed to be manufactured by joining the slabs of the bodies and bases together. Vessel bases became thinner and smoother, indicative of a progressed stage of production. The firing temperature was higher compared to previous periods. Sand appeared as the new tempering agent. The bottle-necked '*fu*' was now absent, with a new type of round-based pot with a tall neck and broad shoulder appearing (Fig. 2-8; Chinese Academy of Social Science *et al.,* 2003).

The major characteristic of Phase V is an impressive development of pottery production. The quantity, typological variety, color and decoration motifs all increased or diversified significantly. Vessels shapes comprised *fu*, pots, basins, open bowls, and plates and cups with high round foot (Fig. 2-8). Crushed calcite was still the major tempering agent, but the grains were smaller and in similar sizes, illustrating they were sieved or ground. Fine pottery and the use of the potter's wheel also occurred and the firing temperatures were higher, however, no excess of 850℃. Decorative motifs included cord texture, string pressing, various combinations of incisions, as well as stamped and impressed patterns (Chinese Academy of Social Science *et al.,* 2003).

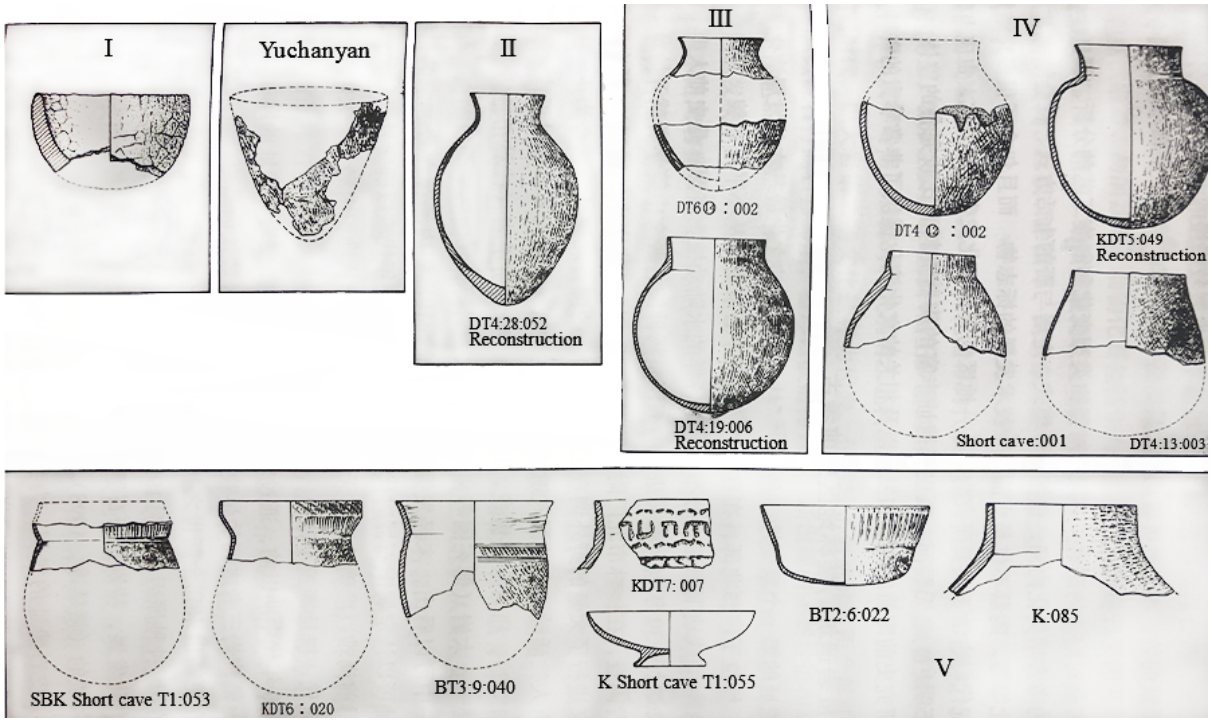


Figure 2-8 Representative reconstructed pottery vessels spanning from the Phase I to V (modified from Chinese Academy of Social Science *et al.,* 2003). Only one shape of pottery is observed in Phase I, and the vessels become taller and thinner in Phase II. A new style of round-based pot with a short neck and large mouth appeared in Phase III. More complex forms emerged in last two phases.

Although pottery-production occurred in the Zengpiyan culture quite early, towards its context, the development was quite static in terms of typological diversities and techniques. It has been theorized that as bamboo and wood cooking or storage wares were used in South China, the pottery industry might have been restricted (Bar-Yosef *et al.,* 2012). Moreover, shellfish and deer were always the staple dietary food in the local human diet during the Zengpiyan period, which means their main dietary habitats would have changed very little through time. If that was the case, there would have been little need to change the type of cooking pottery vessels (Chinese Academy of Social Science *et al.,* 2003).

2.4 Aims of the thesis

As a tentative archaeological research approach, organic residue analysis has already helped to reveal subsistence practises and human diet in ancient Europe, the Near East, and Africa. This technique provides a valuable opportunity to examine, for the first time, diet and subsistence practices in Neolithic China, particularly as understanding of the dietary practices and pottery function is limited, despite the thousands of Neolithic sites found across China. According to the archaeological data to date, it is suggested that the earliest pottery served as cooking devices. Previous ORAs investigating the earliest pottery in Europe suggests that vessels were used to process the products of domesticated animals, while in Japan, people may have needed more durable containers to cook marine products (Evershed *et al.,* 1991a, 1994, 2002b; Craig *et al.,* 2013; Whelton e*t al.,* 2018). Depending on the indigenous resource in each area, thus pottery was invented to process different plant and animal products. The role of pottery vessels in facilitating the processing and storage of wild or cultivated food in China needs to be investigated.

The exploitation of natural resources underpins subsistence in hunting and gathering Neolithic communities. However, in many Chinese sites, early evidence from plant pollen or animal skeletal remains is scarce or inconclusive, and there could be significant ambiguities about timing and intensity. Current methods employed in Chinese Neolithic archaeology are primarily based on ceramic typology, while the reconstruction of diet relies heavily on direct processing evidence, i.e. the phytolith or pollen found within cooking pottery vessels or charred plant remains in pits, hearths. This thesis presents the results of the application of a suite of ORA techniques to investigate the subsistence practices in Zengpiyan targeting the lipids absorbed into the walls of unglazed ceramic pottery during processing or storage. These include lipid residue analysis of potsherds using a combined biomolecular and stable isotopic approach. For the first time, ORA is applied to a Chinese Neolithic site to illustrate the potential that exists for the application of organic residue analysis.

The specific objectives of this thesis are as follows:

1. Investigate the local diet by performing lipid residue analysis on archaeological potsherds obtained from Zengpiyan. Recovering absorbed lipid residue from potsherds to investigate the type of processed products (e.g. animal and plant products, terrestrial versus aquatic) using gas chromatography (GC) and GC-mass spectrometry (GC-MS).

2. Perform compound-specific stable carbon isotope analysis of extracted lipid residues using GC-combustion-isotope ratio-MS to determine the types of animal product consumed (ruminant or non-ruminant animals, terrestrial and aquatic, C_3 versus C_4).

3. Integrate the organic residue results obtained with previous archaeological data and multi-principle studies of potsherds to refine the knowledge of diet and subsistence practices of the ancient people of Zengpiyan.

3. Materials and methods

3.1 Archaeological potsherds samples

A total of 58 potsherds were supplied for organic residue analysis. Potsherds were recovered from different cultural layers during archaeological fieldworks carried out in the 1970 or 2001 excavations at Zengpiyan, China.

Potsherds labelled ZPB and ZPD excavated in 2001 were accompanied by details of phases and pit locations. The fragile fabrics crumbled readily and were prone to breakage making surface cleaning difficult. ZP potsherds were wrapped in foil, but lacked detailed descriptions, making their precise provenance within the excavation unclear. After selection in 2008 potsherds were stored in a deep freeze at -20°C until analysis in 2018/19. Compound-specific radiocarbon dating approach will be carried out to confirm the age of lipid-rich potsherds. The original sample codes allowed matching the potsherds to location described in the institute of Archaeology, Chinese Academy of Social Science *et al.,* (2003) site report. Potsherds' details are listed below:

Table 3-1 List of potsherds in the site report (adapted from Chinese Academy of Social Science *et al.,* 2003).

Potsherd code	Phase	Information
ZPB0001	IIII	'*fu*', bottom
ZPD0005	IIII	Pot, neck or shoulder
ZPD0009	IIII	'*fu*' or pot, bottom
ZPD0014	II	Pot, bottom
ZPD0015	II	Pot, near bottom
ZPD0016	I	Pot, neck or shoulder
ZPD0017	III	'*fu*', neck
ZPD0018	I	'*fu*'

Initial lipid residue analysis of 10 potsherds from Zengpian were conducted by Lucy Cramp utilizing solvent extraction. The rate of recovered lipids was 70%, with mean concentration 22.28 μg g^{-1} and highest concentration of 64.02 μg g^{-1}. The remainder of these 10 potsherds were mainly powder the weight of which was much less than 2 g preferred for organic residue analysis. However, there was still sufficient to undertake direct methanol extraction in this thesis.

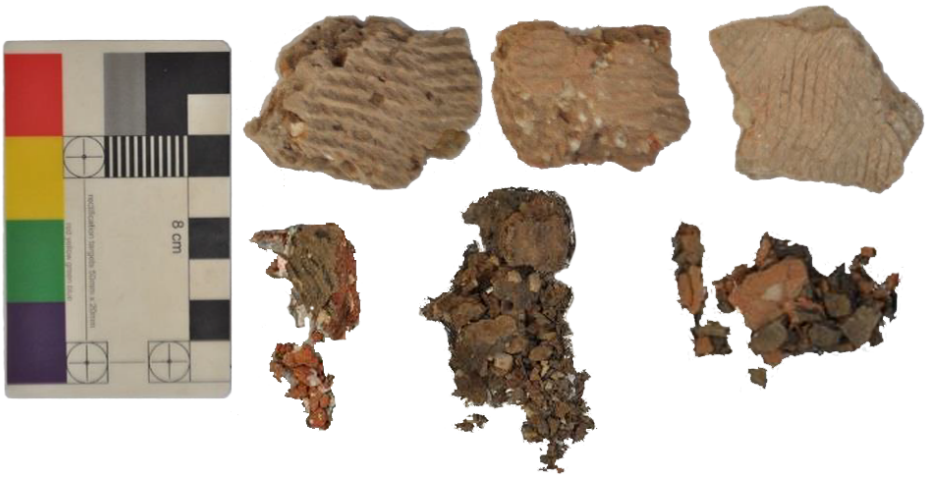


Figure 3-1 Partial ZP and ZPD/ZPB potsherds

The presence and location of soot deposits and fire-clouds on the exterior surface and base of a vessel are clear indicators of usage in cooking or other activities involving fire (Rice, 2015). However, there were no visible external soot or internal charred deposits on any of analysed Zengpiyan potsherds. All samples were body fragments, and most of them possessed thick walls (Fig. 3-1).

3.2 Organic residue analysis methods

3.2.1 General sample handling and preparation

All potsherds were wrapped in aluminum foil and manipulated with tweezers to avoid any external plasticizer or direct-handling contamination after excavation. Some of sherds only comprised powder, which were not usable as they could not be cleaned.

All glassware was cleaned using Decon 90, rinsed with acetone and dichloromethane (DCM), then oven dried and furnaced at 450 °C for 2h prior to use. All solvent used were HPLC or Analytical Grade Standard (typically >98% Purity). Analytical blanks were prepared alongside each batch of samples (usually 10 samples) to act as controls.

3.2.2 Lipid extraction and derivatisation of lipid residues from potsherds

3.2.2.1 Direct acid methanol extraction

All potsherds were cleaned with an electric hand-drill to remove surface contamination then a ca. 2g portion was ground to a fine powder using a mortar and pestle. Twenty microliters of internal standard (C_{34} *n*-alkane, 1 mg mL^{-1} solution) and 5 ml of H_2SO_4/MeOH 4% ($\delta^{13}C$ value measured) were added to each powdered sample contained in a stoppered furnaced culture tube

(I), which was then placed on a heating block (70 °C, 1 h) with whirl mixing every 10 min. Once cooled, the sulfuric acid-methanol supernatant was transferred to a clean test tube and centrifuged (2500 rpm,10 min). The supernatant was removed to another clean culture tube (II) mixed with additional 2ml (DCM) extracted double-distilled water. A further 2 x 3ml *n*-hexane was added to the original potsherd powder to recover any extracted lipids not fully solubilised into acidified methanol, mixed well and transferred to culture tube (II). Culture tube (II) was whirlimixed and the TLE (hexane, top layer) transferred to a 3.5ml vial. The *n*-hexane solution was gently blown down under nitrogen on a heating block at 40 °C. A further 2 x 2ml of *n*-hexane was added to the acidified methanol solution and the whirlimixing transfer to the same 3.5 vial and blowing down repeated. The TLE was kept in a fridge until required for derivatization and instrumental analysis.

An aliquot of the lipid extract was transferred into another 3.5ml vial, blown down under a gentle stream of nitrogen in a heating block at 40 °C. The dried TLE was then trimethylsilylated with 20 µl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; 70°C, 1h), then blown down to dryness under nitrogen. The lipid extract was diluted with 50 or 100 µl *n*-hexane prior to analysis by GC, GC-MS, and GC-C-IRMS.

3.2.2.2 Solvent extraction

Approximately 2g of a cleaned portion of potsherd was crushed using a mortar and pestle and placed into a furnaced 28ml vial with 20 µl of internal standard solution (C_{34} *n*-alkane, 0.1mg ml^{-1}). A mixture of chloroform/methanol (2:1 *v/v;* 2 x 10 ml) was used to extract the absorbed lipids aided by 20 min sonication. The solution was centrifuged (2500 rpm, 10 min) and the supernatant decanted into a 3.5ml vial. The solvent blown down under nitrogen, gradually topping up the vial until a full vial of chloroform/methanol remained. The TLE was stored in a fridge until required for instrumental analysis.

BSTFA (40 µl) was added to the filtered (silica gel) and dried aliquot of TLE, then heated for 1h at 70°C. Blow down the derivatizing agent and dilute the extract with 100µl *n*-hexane for analysis by HTGC and HTGC-MS.

3.2.3 Instrumental analysis

3.2.3.1 Gas chromatography (GC, HT-GC)

3.2.3.1.1 Agilent 7820A

GC analysis of acid methanol extracted TLEs was carried out using an Agilent Technologies 7820A gas chromatograph fitted with the autosampler injector and flame ionisation detector (FID). The derivatised TLEs (1 μL) were introduced to the system by an on-column injection. The oven was equipped with a 50 m × 0.32 mm (Agilent J&W Scientific) fused silica capillary column coated with a 100 % dimethylpolysiloxane HP-1 non-polar stationary phase (0.17 μm film thickness). The GC temperature programme involved an initial 50 °C hold for 2 min after injection, followed by temperature ramping at 10 °C min^{-1} up to 300 °C, and then an isothermal hold for 15 min. Helium was used as the carrier gas maintained at a constant flow (2.0 mL min^{-1}). Data was acquired using Agilent OpenLAB panel and quantification of TLEs was achieved by reference to the known amount of internal standard as described in data processing section below.

3.2.3.1.2 Agilent 7890A

HT-GC analysis of trimethlsilylated solvent extracted TLEs was carried out using an Agilent Technologies 7890A gas chromatograph fitted with an autosampling injector and flame ionisation detector. The derivatised TLEs (1 μL) were introduced by an on-column injection. The analytical column was a 15 m × 0.32 mm (Agilent J&W Scientific) fused silica capillary column coated with a 100 % dimethylpolysiloxane Rxi-HT non-polar stationary phase (0.1 μm film thickness). The GC temperature programme involved an initial 50 °C hold for 2 min after injection, followed by temperature ramping at 10 °C min^{-1} up to 350 °C, after which the oven was held isothermally for 10 min. Helium was used as the carrier gas maintained at a constant flow (4.26 mL min^{-1}). Data was acquired using HP Chemstation software (Rev. C.01.07 [27] Agilent Technologies) and quantification of TLEs was achieved by reference to the known amount of added internal standard as described in the data processing section.

3.2.3.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis of the methanol acid extracted TLEs was carried out using a ThermoScientific Trace 1300 series gas chromatograph, equipped with an AI/AS 1310 injector, coupled to an ISQ single quadrupole mass spectrometer. The PTV injector was set to splitless mode (split flow 12.0 mL min^{-1}, split ratio 6.7). The analytical column was a 50 m x 0.32 mm (Agilent

J&W GC Columns) fused silica capillary column coated with a 100 % dimethylpolysiloxane HP-1 non-polar stationary phase (0.17 μm film thickness). The GC temperature programme involved an initial 50 °C hold for 1 min after injection, followed by temperature ramping at 10 °C min^{-1} up to 300 °C, the oven was then held isothermally for 8 min. Helium was used as the carrier gas maintained at a constant flow (2.0 ml min^{-1}). The MS was operated in the electron ionization (EI) mode with the electron energy set at 70 eV and emission current set to 50 μA. The MS transfer line was set at a temperature of 300 °C and ion source temperature at 300 °C. The MS was set to scan *m/z* 50-650 at 1.7 scans s^{-1} in full-scan mode. Identification of common lipids was performed by comparison with the NIST mass spectral database or mass spectra from the published literature.

For detection of DYHA the MS was operated under the exact same conditions as above but with selected ion monitoring (SIM) mode using the ions *m/z* 215, 243, 259, 287, 443, 489, 471, 487, 499, and 515. The data acquisition and processing were carried out using XCalibur software (version 3.0 SP2, ThermoScientific).

GC-MS analysis to detect APAAs was carried out using a ThermoScience Trace 1300 Series GC, coupled to an ISQ single quadrupole mass spectrometer and an AI/AS 1310 autosampler injector. The PTV injector was set to splitless mode (split flow 10.0 mL min^{-1}, split ratio 6.7). The analytical column was a 60m x 0.32 mm (Agilent J&W Scientific) fused silica capillary column coated with a 50% cyanopropyl-methylpolysiloxane VF-23ms polar column stationary phase (0.15 μm film thickness). The GC temperature programme began with an isothermal hold at 50 °C for 2 min after injection, followed by temperature ramp of 10 °C min^{-1} up to 100 °C and subsequently 4.0 °C min^{-1} up to 240 °C, the oven was then held isothermally for 15 min. Helium was used as the carrier gas maintained at a constant flow (2.0 ml min^{-1}). The MS was operated in the EI mode with electron energy set at 70 eV and emission current at 50 μA The MS transfer line temperature was set to 260 °C and ion source temperature to 240 °C. The MS was set to scan *m/z* 50-650 at 1.7 scans s^{-1} in full-scan mode and m/z 105, 262, 290, 318 and 346 in SIM mode. The GC-MS data acquired was processed using XCalibur software (version 3.0 SP2, ThermoScientific). Identification of APAAs was performed by comparison with the NIST mass spectral database or reference GC-MS data from published analyses (Cramp and Evershed, 2014).

GC-MS detection of isoprenoid acids was carried out using a ThermoScience Trace 1300 Series GC, using manual injections, coupled with an ISQ single quadrupole MS. The PTV injector

was set to splitless mode (split flow split flow 10.0 ml min^{-1}, split ratio 6.7). The analytical column was a 60 m x 0.32 mm (Agilent J&W Scientific) fused silica capillary column coated with a 50% cyanopropyl-methylpolysiloxane VF-23ms polar column stationary phase (0.15 μm film thickness). The GC temperature programme involved an initial hold at 50 °C for 2 min after injection, followed by temperature increase rate of 10 °C min^{-1} up to 240 °C, followed by an isothermal hold for 10 min. Helium was used as the carrier gas and maintained at a constant flow (2.0 ml min^{-1}). The MS was operated in the EI mode with the electron energy set at 70 eV and emission current at 50 μA. The MS transfer line temperature was held at 260 °C and ion source temperature at 240°C. The MS was set to scan *m/z* 50-650 at 1.7 scans s^{-1} in full-scan mode and m/z 87, 88, 101, 270, 312 and 326 in SIM mode. Data acquisition and processing were carried out using XCalibur software (version 3.0 SP2, ThermoScientific). Identification of isoprenoids was performed by comparison with the NIST mass spectral database or reference GC-MS data from the published literature.

HTGC-MS analysis of the solvent extracted TLEs for the detection of waxes was carried out using a ThermoScience Trace 1300 Series GC, equipped with an AI/AS 1310 autosampler injector, coupled to an ISQ single quadrupole MS. The PTV injector was set to split mode (split flow 30.0 mL min^{-1}, split ratio 6.0). The oven was equipped with a 15m x 0.32mm (Agilent J&W Scientific) fused silica capillary column coated with a 100% dimethylpolysiloxane DB-1HT high-temperature stationary phase (0.10 μm film thickness). The GC temperature programme involved an initial 50.0 °C for 2 min after injection, followed by temperature ramping at 10.0 °C min^{-1} up to 280 °C, subsequently 25.0 °C min^{-1} up to 380 °C, the oven was then held isothermally for 5min. Helium was used as the carrier gas maintained at a constant flow of 5.0 mL min^{-1}. The MS was operated in the EI mode with the electron energy set at 70 eV and emission current set to 150 μA. The MS transfer line was set at a temperature of 380 °C and ion source temperature at 340°C. The MS was set to scan *m/z* 50-950 at 2 scans s^{-1} in full-scan mode. The GC-MS data acquired was processed using XCalibur software (version 3.0 SP2, ThermoScientific). Identification of lipids was performed by comparison with the NIST mass spectral database or reference GC-MS data from the published literatures.

3.2.*3*.3 Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS)

Compound-specific carbon stable isotope analysis was carried out on an Agilent Industries 7890A gas chromatograph, using Agilent7890 autosampler injector, coupled with an IsoPrime 100 mass spectrometer. The column oven was equipped with a 50 m x 0.32 mm (Agilent J&W

Scientific) fused silica capillary column coated with a 100% dimethylpolysiloxane HP-1 non-polar column stationary phase (0.17µm film thickness). The GC oven temperature programme involved an initial hold at 40 °C for 2 min after injection, followed by temperature ramp of 10 °C min^{-1} up to 300 °C, the oven was then held isothermally for 10 min. Helium was used as a carrier gas maintained at a constant flow of 2.0 ml min^{-1}. The combustion interface was a quartz tube filled with CuO pellets maintained at a temperature of 850 °C. Instrument accuracy was monitored by use of an external FAME standard mixture (C_{11}, C_{13}, C_{16}, C_{21} and C_{23}) of known isotopic composition, analysed every 4 runs of unknown samples. Instrument error was ± 0.3 ‰. Samples were run in duplicate and an average taken. The $\delta^{13}C$ values are the ratio of $^{13}C/^{12}C$ expressed relative to the international standard Vienna Pee Dee Belemnite (VPDB), calibrated against a reference CO_2 gas of known isotopic composition.

3.2.4 Data processing

3.2.4.1 Quantification of extracted lipids

The quantification of recovered lipids was calculated by reference to the internal standard (*n*-tetratriacontane, C_{34} *n*-alkane). All samples were added 20 µg internal standard. The quantification was calculated as follows:

$$M_{lipid} = 100 \times \frac{M_{IS}}{A_{IS}} - \frac{M_{IS} \times A_{cont}}{A_{IS}} - M_{IS}$$

Where:

M_{lipid} = mass of lipids presented in the TLE

M_{IS} = mass of the added internal standard (typically 20 µg per sample)

A_{IS} = peak area of internal standard (in %)

A_{cont} = total peak areas of contamination (in %)

The concentration of absorbed lipid residues was calculated as follows:

$$C_{lipid} = \frac{M_{lipid}}{M_{potsherd\ powder}}$$

Where:

C_{lipid} = concentration of the TLE

M_{lipid} = mass of the TLE

$M_{potsherd\ powder}$ = mass of potsherd powder

3.2.4.2 $\delta^{13}C$ values

The $\delta^{13}C$ values ($^{13}C/\ ^{12}C$) were calculated as follows:

$$\delta^{13}C_{sample} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

Where:

$\delta^{13}C_{sample}$ = $\delta^{13}C$ values of the FAME (in ‰)

R_{sample} = $^{13}C/^{12}C$ in the FAME

$R_{standard}$ = $^{13}C/^{12}C$ in the standard

The $\delta^{13}C$ values measured for each fatty acid methyl ester was corrected to remove the exogenous carbon added through methylation. The determination of the corrected $\delta^{13}C_{FA}$ values was performed according to the following mass balance equation (Rieley, 1994):

$$\delta^{13}C_{FA} = \frac{(n+1)\delta^{13}C_{FAME} - \delta^{13}C_{CH3OH}}{n}$$

Where:

n = total numbers of carbon atoms

$\delta^{13}C_{FA}$ = $\delta^{13}C$ value of the original fatty acid (in ‰)

$\delta^{13}C_{FAME}$ = measured $\delta^{13}C$ value of the FAME (in ‰)

$\delta^{13}C_{CH3OH}$ = measured $\delta^{13}C$ value of CH_3OH used for methylation

4. Results and discussion

4.1 ORA of pottery from Zengpiyan

ORA of Neolithic potsherds has been conducted in many different regions of the world (e.g. Europe, Africa, America, and the Near East) to investigate local subsistence practices (Eerkens, 2001, 2005; Evershed *et al.,* 2008b; Dunne *et al.,* 2012, 2017; Roffet-Salque *et al.,* 2013). In Asia, the analyses have been performed on archaeological potsherds from Japan on Late Pleistocene Jomon pottery, to investigate vessel function, as well as differentiating terrestrial from aquatic food procurement and processing (Craig *et al*. 2013; Lucquin *et al.,* 2016b). ORA provides direct evidence for pottery use, however, as discussed above the approach has been little used for Chinese ancient pottery yet (Section 1.3).

In this thesis the first systematic results of ORA of Chinese Neolithic pottery are presented. The results are presented first as a brief summary, Section 4.1.1, followed by sections providing more in depth interpretations and discussions in relation to other archaeological information from Zengpiyan specifically: (i) terrestrial animal exploitation, (ii) plant processing, including diet and technological uses, and (iii) the question of aquatic resource exploitation.

4.1.1 Summary of analyses of GC, GC-MS and GC-C-IRMS analyses

A total of 58 Neolithic potsherds from Zengpiyan were analysed in this thesis. Thirty three percent of analysed potsherds contained interpretable amounts of lipids (lipid concentration>5μg g^{-1}) with a mean lipid concentration of 0.22 mg g^{-1}, the highest concentration was 1.4 mg g^{-1}. It has been suggested that pottery at Zengpiyan was used for processing shellfish (Chinese Academy of Social Science *et al.,* 2003), however, the relatively low lipid recovery rate, compared with the analysis of pottery from the Jōmon site of Torihama in western Japan where a recovery rate of 80-94% was seen for different periods (Lucquin *et al.,* 2016b), may point to the use of non-pottery containers, e.g. bamboo for cooking and storage (Bar-Yosef e*t al.,* 2012).

All eluting peak assignments were confirmed by GC-MS (mass spectra are listed in Appendix 2). The most common lipid distribution recovered from Zengpiyan potsherds with significant concentrations was dominated by a high abundance of *n*-$C_{16:0}$ (P) and *n*-$C_{18:0}$ (S) fatty acids (*n*=10, 52.63%), ambiguously assigned as degraded animal products (Table 4-1). Odd-numbered (*n*-$C_{15:0}$ and *n*-$C_{17:0}$) and their branched-chain (*iso*- and *anteiso-)* counterparts were

also present in 14 extracts, possibly being indicative of bacterial origin or diagnostic of ruminant animal fats (Dudd and Evershed, 1998; Evershed *et al.,* 2002b).

These extracts were selected for GC-C-IRMS analysis to determine the $\delta^{13}C$ values (Table 4-1), diagnostic of non-ruminant and ruminant adipose fats (Dudd and Evershed, 1998; Copley *et al.,* 2003).

Table 4-1 Stable carbon isotope values of absorbed animal fats within Zengpiyan pottery (NRA: non-ruminant fat; RA: ruminant fat; RDA: ruminant dairy fat).

Sherd number	$\delta^{13}C_{16:0}$	$\delta^{13}C_{18:0}$	$\Delta^{13}C$	Classification
ZP0013	-24.5	-27.5	-3.0	RA/RDA
ZP0031	-28.4	-31.3	-2.9	RA/RDA
ZP0033	-27.4	-29.3	-1.9	RA
ZPB0002	-25.6	-27.4	-1.8	RA
ZPB0003	-22.9	-25.2	-2.3	RA
ZPD0005	-28.3	-29.8	-1.6	RA
ZPD0014	-22.7	-21.7	1.0	NRA
ZPD0015	-27.2	-27.0	0.2	NRA/RA
ZPD0017	-28.1	-27.7	0.4	NRA
ZPD0018	-25.9	-26.8	-0.9	RA

Of the TLEs investigated 7 extracts (42%) yielded plant biomarkers, *n*-alkanes or *n*-alcohols, possibly indicative of plant processing in the vessels. Among all the lipid extracts, only one lipid extract (ZP0013) exhibited P/S value ≥1.3, which possibly indicated the processing of both animals and plant products (Mills and White, 1994). LCFAs are biomarkers associated with plant cuticular waxes (Post-Beittenmiller, 1996). Even-over-odd numbered long-chain fatty acids up to *n*-C_{34} in chain length were identified in 15 extracts. Their occurrence in low abundance in animal fats is consistent with from animal products processing (Correa-Ascencio and Evershed, 2014; Whelton *et al.,* 2018). However, here 4 extracts contained unusually high abundance of LCFAs, suggesting an origin from plants rather than animals (higher or almost equivalent to the $C_{16:0}$ or $C_{18:0}$ fatty acid; Table 4-2).

Table 4-2 Summary compositions of lipid extracts of potsherds containing plant biomarkers (*n*-alkanes or *n*-alcohols).

Sherd number	P/S ratio	*n*-alkanes	*n*-alcohols	Plant-derived LCFAs
ZP0001	1.079	C_{24}-C_{27}, C_{29}	C_{24}-C_{30}, C_{32}	C_{20}-C_{33}
ZP0010	1.2	C_{25}, C_{27}	C_{22}-C_{30}, C_{32}	C_{20}-C_{32}
ZP0011	0.65	C_{23}-C_{27}, C_{29}	C_{20}-C_{30}, C_{32}	C_{20}-C_{32}
ZP0013	1.83	-	-	-
ZP0026	-	C_{25}-C_{30}	C_{24}, C_{26}-C_{30}	C_{20}-C_{32}
ZP0031	0.28	-	C_{26}, C_{28}, C_{30}	-
ZP0033	0.55	-	C_{26}, C_{28}, C_{30}	-
ZPB0006	0.59	C_{23}, C_{25}, C_{27}, C_{30}	C_{26}	-
ZPD0013	0.38	-	C_{24}, C_{26}, C_{28}, C_{30}, C_{32}	C_{20}-C_{34}

Except for the lipid profiles dominated by *n*-$C_{16:0}$ and *n*-$C_{18:0}$ fatty acids, the others showed two unusual lipid distributions, one was where the $C_{18:1}$ fatty acid dominated the TLEs. Such distributions have rarely been observed as $C_{18:1}$ is unstable and readily degraded over long archaeological timescales (Copley *et al.,* 2001). All extracts were screened in SIM mode for detecting aquatic biomarkers, isoprenoids, APAAs and dihydroxy fatty acids. C_{18} APAAs were detected in ZP0033, pristanic and phytanic acids in ZPB0003, and 9,10-dihydrostearic fatty acids in 12 extracts. No C_{20} or C_{22} APAAs and longer-chain dihydroxy fatty acids were observed. The detailed results of the analyses of 58 Neolithic potsherds from Zengpiyan are summarized in Appendix 1.

4.2 The exploitation of animal products

The organic residue analyses conducted on 58 Zengpiyan potsherds have confirmed primarily that animal products were processed within pottery vessels. The latter is interpreted based on the dominance of the $C_{18:0}$ fatty acid relative to $C_{16:0}$ fatty acid, whereas in plants the situation is reversed (Mills and White, 1994). A typical gas chromatogram of a degraded animal fat is displayed in Fig. 4-1 showing the significant predominance at *n*-$C_{16:0}$ and *n*-$C_{18:0}$ fatty acids, the latter confirming the lipids originated from the processing of animal products in the vessel. Among the potsherds containing extractable lipid residues, the P/S values ($C_{16:0}$/$C_{18:0}$) ranged from 0.28 to 1.83, suggesting animal origins. The abundance of odd-numbered $C_{15:0}$ and $C_{17:0}$

(even $C_{19:0}$) and their branch-chain *iso*- and *anteiso*-isomers is consistent with a largely ruminant animal origin (Fig. 4-1; Dudd and Evershed, 1998; Evershed *et al.,* 2002b).

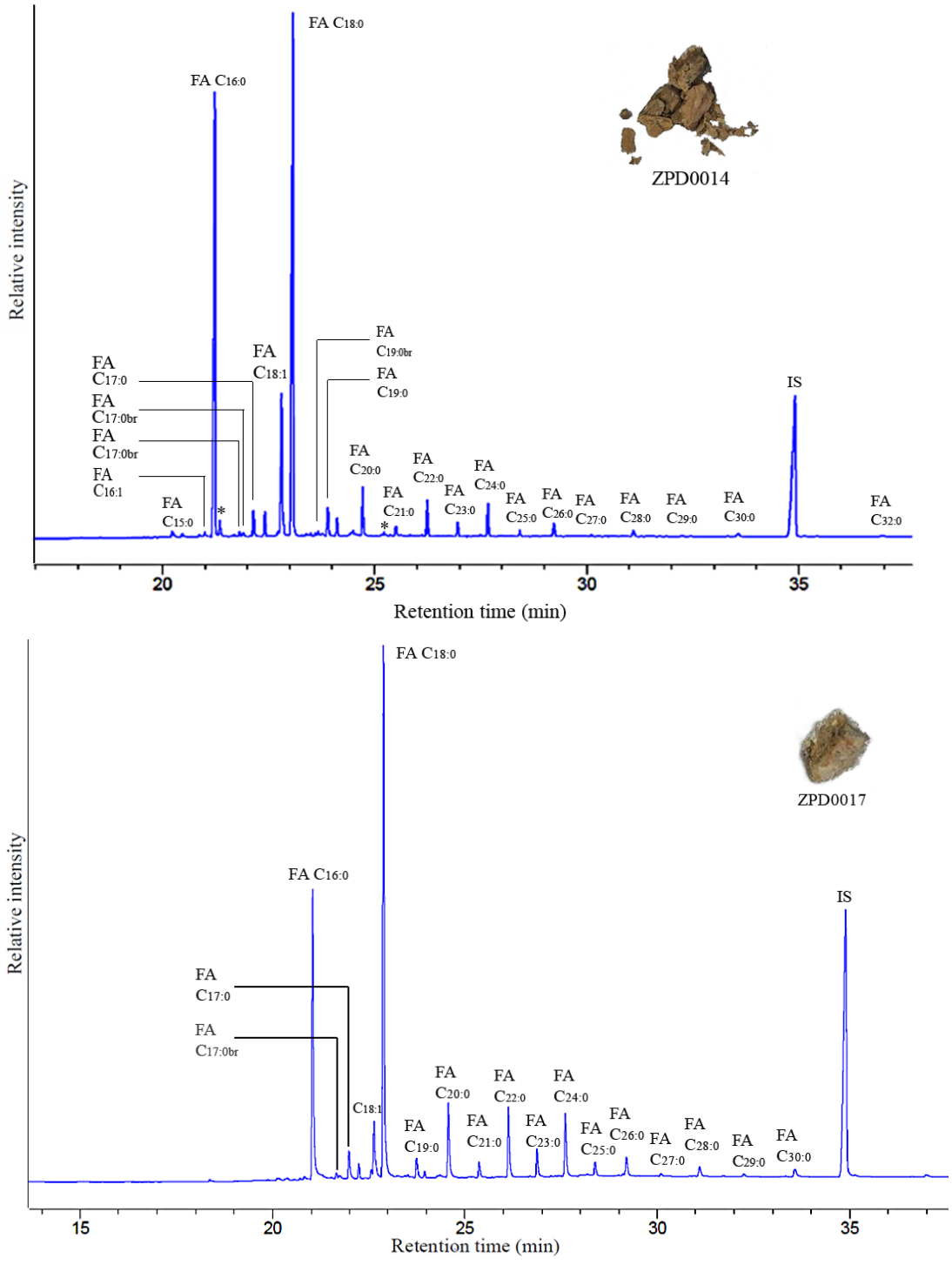


Figure 4-1 Partial gas chromatograms of the TLEs from ZPD0014 and ZPD0017, illustrating the distribution of lipids characteristic of partially degraded animal fats. Key: FA Cx:y are the free fatty acid of carbon number X and Y degrees of unsaturation, br comprises *iso*- or *anteiso*-branched fatty acid. IS is the added internal standard (C_{34} *n*-alkane) and * denotes plasticiser contamination.

4.2.1 Stable carbon isotope analysis

Of those organic residues interpreted as originating from animal products (based on the relatively lower abundance of *n*-$C_{16:0}$ compared to *n*-$C_{18:0}$ fatty acid, Tolloch, 1976), compound-specific carbon isotope analysis was performed to characterise the origin of the fats (Dudd and Evershed, 1998; Copley *et al.,* 2003). The $\delta^{13}C_{16:0}$ values obtained ranged from -28.4 to -22.7‰ and the $\delta^{13}C_{18:0}$ values were between -31.3 and -21.7 ‰. When compared to reference values of ruminant and non-ruminant animals raised on a strict C_3 diet, the carbon isotopic values from Zengpiyan exhibited higher $\delta^{13}C$ values, plotting in the range from ruminant to non-ruminant and aquatic fats (Fig. 4-2; Copley *et al.,* 2003). Most of the extracts plotted between the reference non-ruminant or ruminant adipose fats ellipses, suggesting either the mixture of different animal fats or involved the processing plants contemporaneously or during the lifetime of use of vessels.

Plotting the $\Delta^{13}C$ (= $\delta^{13}C_{18:0}$ - $\delta^{13}C_{16:0}$) values (Fig. 4-2) removes environmental effects on the classification of animal fats, emphasizing the difference in biosynthesis and metabolism (Copley *et al.,* 2003). The majority of the TLEs plotted within the ranges represented ruminant adipose fats (50.0%) and non-ruminant adipose/plant products (20.0%) or a mixture of two (10.0%). A further two plotted on the edge of ruminant adipose or dairy fats occupied 20.0%. None of the residues fell into the range of dairy fats (Fig. 4-2). The geographical location of Zengpiyan is not coastal, thus, the assumption of marine influence was eliminated (Chinese Academy of Social Science *et al.,* 2003). In the $\Delta^{13}C$ values plot the $\delta^{13}C_{16:0}$ values varied over a wide range (-28.4 to -22.7‰), inferring the enrichment in $\delta^{13}C$ was influenced by animals whose diets comprised a significant proportion of C_4/marine inputs with addition of partial C_3 plants.

The practicing of meat subsistence strategies at the site is suggested by the $\Delta^{13}C$ values, which ranged from -3.0 to 1.0‰, indicating that vessels were intensively used to process ruminant/non-ruminant carcass products. The subsistence practices in Zengpiyan have previously been interpreted to be meat-based, as indicated by the substantial amount of mammalian remains unearthed from Zengpiyan, identified as abandoned food waste (Chinese Academy of Social Science *et al.,* 2003). The discovery of abundant fatty acids of mixed animal origin is consistent with the zooarchaeological finds at Zengpiyan.

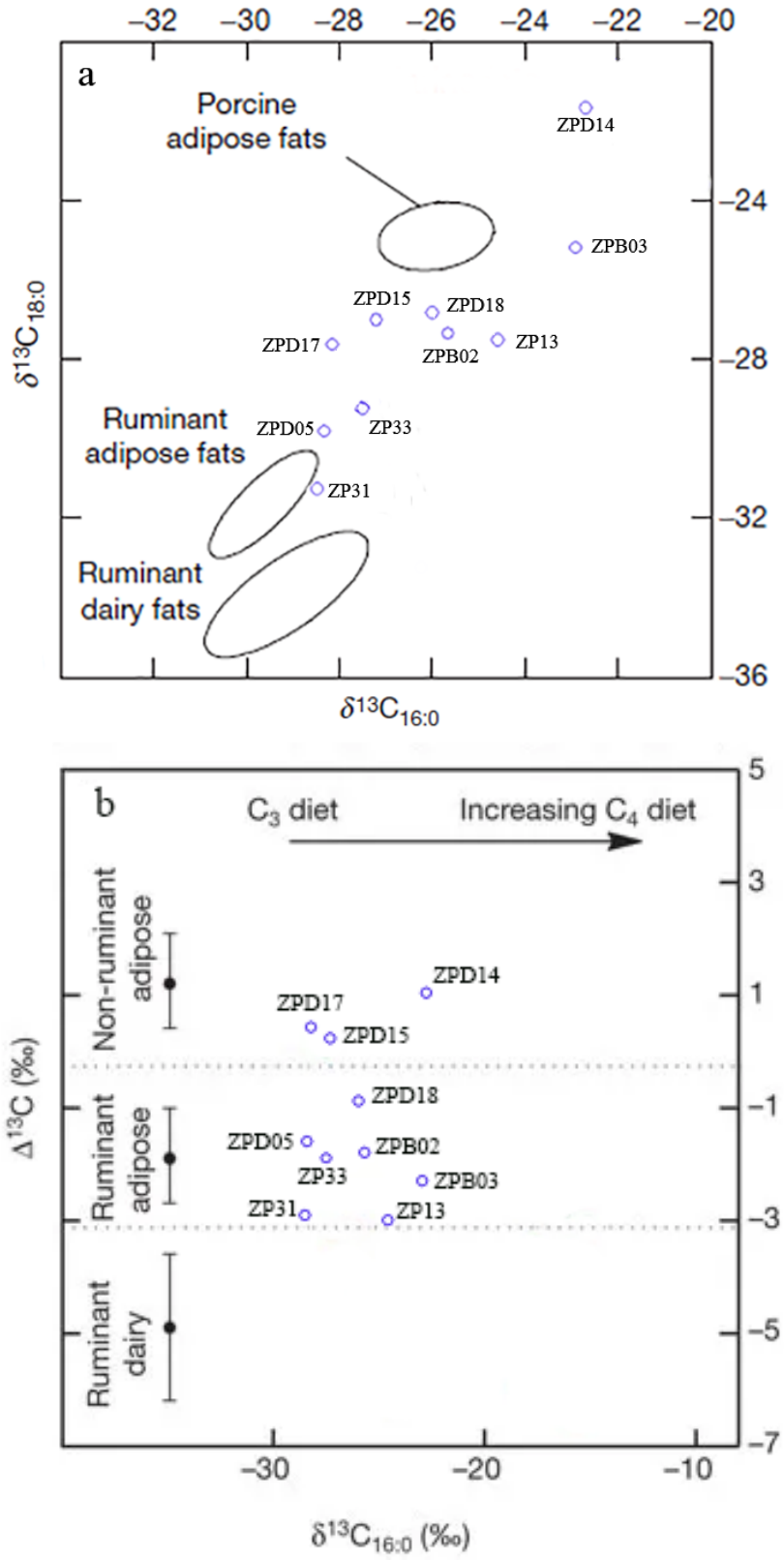


Figure 4-2 a) Scatter plot showing the $\delta^{13}C$ values of $C_{16:0}$ plotted against $C_{18:0}$ fatty acids from lipid extracts from Zengpiyan; b) Scatter plot of $\Delta^{13}C$ ($\delta^{13}C_{18:0}$ - $\delta^{13}C_{16:0}$) values plotted against $\delta^{13}C_{16:0}$. The $\delta^{13}C$ values of modern reference fats were adjusted for post-Industrial Revolution effects of fossil fuel burning by the addition of 1.2‰ (Friedl *et al.,* 1986). The confidence ellipses represent the $\delta^{13}C$ values of modern reference animals in Britain (raised in a pure C_3 diet), Africa, Kazakhstan, Switzerland and the Near East (Copley *et al.,* 2003; Dunne *et al.,* 2012).

Concerning specific meat sources, from faunal assemblage of Zengpiyan, deer and pigs comprise *ca.* 87% of mammal remains, with pig the only non-ruminant animal recovered from mammal assemblages (Chinese Academy of Social Science *et al.,* 2003). As the possibility of marine sources were eliminated and those residues likely of plant origin being not selected for isotopic analysis, the $\Delta^{13}C$ values of 2 extracts (ZPD0014, ZPD0017) indicated the origin of non-ruminant animals, most likely pigs. ZPD0015 was likely to derive from a mixture of ruminant and non-ruminant carcass products. The determined ages at death of the excavated pig fragments were all under 2 years old (Chinese Academy of Social Science *et al.,* 2003), the human inhabitants were consciously targeting younger animals, however, neither the lipid nor faunal remains is sufficient to suggest evidence of pig husbandry.

4.2.1.1 Evidence of C_4 plants in the Zengpiyan ecosystem

The $\delta^{13}C$ values of individual fatty acids $C_{16:0}$ and $C_{18:0}$ varied over a wide range (-28.4 to -22.7‰ for the $\delta^{13}C_{16:0}$ values and the $\delta^{13}C_{18:0}$ values were between -31.3 and -21.7 ‰), indicating that various plant species existed in the environment. Several of the extracts corresponding to degraded animal fats, yielded carbon isotope values indicating that the animals had consumed a significant proportion of C_4 plants (ZPB0003 and ZPD0014; Copley *et al.,* 2003; Roffet-Salque, 2012; Dunne *et al.,* 2012). Indeed, from the palaeobotanical evidence recovered from Zengpiyan, wild C_4 vegetation appeared to substantial (see below), moreover, traces of CAM plants were also found (Fig. 4-4; Chinese Academy of Social Science *et al.,* 2003). Residue extracts with much higher carbon isotopic values (ZPB0003 and ZPD0014) appeared to have originated from an animal fat source. There is no evidence of mixing with plant resources in these vessels due to the lack of plant biomarkers (their LCFAs are presumed to have arisen *via* animal dietary vegetations and are not directly plant-derived; Halmemies-Beauchet-Filleau *et al.,* 2013, 2014; Whelton *et al.,* 2018). Based on a modified proxy, the observed results suggest the wild animals subsisted on a C_3 plant diet with varying addition of some C_4 resources (Fig. 4-3; Roffet-Salque, 2012; Dunne *et al.,* 2012).

Given the influence of marine fats been eliminated based on Zengpiyan's geographic location, the extract of ZPD0014 plotted in marine reference ellipse, however, as illustrated above the influence of a combined C_3 and C_4 diet would move residues to this position on the plot.

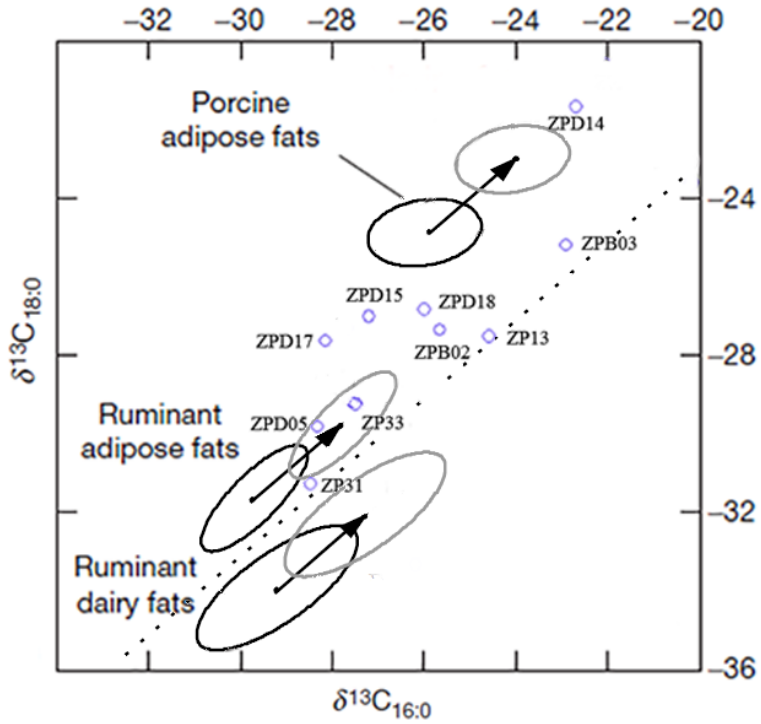


Figure 4-3 Scatter plot showing the $\delta^{13}C$ values of $C_{16:0}$ plotted against $C_{18:0}$ fatty acids from Zengpiyan extracts plotted within confidence ellipses from modern reference fats raised on a strict C_3 diet in Britain (P=0.684; Copley *et al.,* 2003) and hypothesised confidence ellipses for animals raised on a mixed C_3/C_4 diet (Roffet-Salque, 2012).

Archaeobotanical evidence obtained from the sieving the sediments in the 2001 excavation indicate an abundance of C_4 plants existed during Neolithic times. Moreover, it is notable that C_4 edible plants, such as mock strawberry (fruit; *Duchesnea indica*), shepherd's purse (*Capsella bursa-pastoris*), Cherokee rose (fruit; *Rosa Iaevigata*) and Chinese hackberry (fruit; *Celtis sinensis*) were widely observed in this region, which can be consumed by human or animals (Fig. 4-4; Chinese Academy of Social Science *et al.,* 2003).

Figure 4-4 Examples of edible C_4 plants lived in Zengpiyan, from left to right, mock strawberry (fruit); shepherd's purse; Cherokee rose (fruit); Chinese hackberries (fruit; modified from Chinese Academy of Social Science *et al.,* 2003).

Aside from the influence of vegetation species, consideration of broad range of the $\delta^{13}C$ values from potsherds was akin to previously studies performed on northern Greece and Takarkori, Africa (Dunne *et al.,* 2018; Whelton *et al.,* 2018). The variations in $\delta^{13}C$ values could suggest seasonal movements between different ecosystems of the wild animals (Dunne *et al.,* 2018;

Whelton *et al.,* 2018). For example, in montane regions, nomadic societies practice vertical transhumance impling the difference of pastural growth temperature, which affect the $\delta^{13}C$ values of the plant carbohydrates and fatty acids by 0.4‰ every 1 ℃ (DeNiro and Epstein, 1977). As there is no confirmed domestication or evidence of pastoralism at Zengpiyan period, it is as yet impossible to answer the question whether any seasonal subsistence practices existed. However, Zengpiyan site is located in the montane region on the fringes of the Dushan Mountain, which is up to approximately 250 meters. The dietary compositions of wild animals can be shifted from the mountains to the plain (Chinese Academy of Social Science *et al.,* 2003).

Likewise, environmental factors also affect $\delta^{13}C$ values of C_3 plants which feeds through to the consumers tissues, such as Neolithic sheep grazing in salt marshes (Balasse *et al.,* 2006; Schulting *et al.,* 2017), drought-stressed conditions observed in Europe (Evershed *et al.,* 2008b; Mukherjee *et al.,* 2005) and the high temperature, observed in Africa (Dunne *et al.,* 2012). Current data suggest plant taxa are diverse in Zengpiyan region. Thus, it seems the variations in $\delta^{13}C$ values seen in the animal fats in pots arose from animals taking different pastures or combination of C_3 and C_4 plants in the diets of the consuming animals (Chinese Academy of Social Science *et al.,* 2003; Guo *et al.,* 2015).

4.2.1.2 Deer as a dietary component

In terms of the $\delta^{13}C$ values of animal fats compared with reference ruminant adipose fats, the broader range of these $\delta^{13}C$ values were possibly been affected by extensive processing of deer products within pottery vessels. Unlike domesticated ruminant animals, deer processing within antiquity have been rarely investigated. It is clear from the faunal remains that the occupants in Neolithic Zengpiyan specialized in hunting two species deer, sika (*Cervus nippon*) and muntjac (*Cervus muntjac*) deer, which constituted more than 80% of the hunted mammals (Chinese Academy of Social Science *et al.,* 2003). Habitat compositions of sika deer are quite flexible, being strongly driven by the available vegetations, including forests, marshes, and grasslands. This deer species also migrates seasonally to montane regions, with winter ranges being up to 700m lower in elevation than the summer ranges (Whitehead, 1993). Sika deer are therefore highly environmentally adaptable, with their diet concentrating on graminoids in winter, forbs and crops in spring and summer and foraging all these plant foods in autumn (Yokoyama *et al.,* 2000).

Muntjac deer also span a broad range of eating habits, with bramble and raspberry being their most important foods and grasses prevailing in spring and shrubs, trees and forbs in the rainy seasons (Nagarkoti and Thapa, 2007). In China, muntjac deer are predominantly browsers rather than grazers (Jackson and Chapman, 1977).

Nearly half of all grass species exhibit C_4 photosynthesis (Sage, 2004). In Zengpiyan region, under a consistent tropical/subtropical temperate, the number of C_3 grass species is almost equal to C_4 ones (Ge, 2009). In graze seasons, grass will dominate in the diet of muntjac or sika deer. *Imperata cylindrical*, *Pogonatherium paniceum, Saccaharum spontaneum,* these C_4 grasses will be taken in significant amounts with other C_3 grasses, thus, the $\delta^{13}C$ values of deer fats intermediates C_3-C_4 reference values.

Also, such relatively mobile lifestyles of these animals, probably with long-distance movement to various pastures (ranging from dry or humid environment, or low or high altitudes), will lead to a wide range of carbon isotope values (Dunne *et al.,* 2018; Whelton *et al.,* 2018). Moreover, even when deer are raised on a constant diet, deer adipose fats seem to exhibit a greater range of carbon isotopic values than other animals feeding on the same diet, even reaching the $\Delta^{13}C$ range of dairy fats (e.g. Evershed *et al.,* 2002a; Craig *et al.,* 2012). The above feeding traits of deer likely account for the enriched $\delta^{13}C$ values of the fatty acids seen in the pot lipid extracts, suggesting extensive processing of deer products in the Zengpiyan pottery.

4.2.2 Long-chain fatty acids (LCFAs) in animal-derived residues

Among these animal-derived residues, the interpretation become complex due to the high abundance of even carbon numbered LCFAs. Aside from *n*-$C_{16:0}$ and *n*-$C_{18:0}$ fatty acids, 15 extracts yielded LCFAs and 5 of them were present in too low abundance to determine their $\delta^{13}C$ values, others exhibited carbon isotope values falling into the ruminant and non-ruminant adipose fats categories. The obvious interpretation of the origin of the LCFAs in the extracts is that they derive from processing plants (Tulloch, 1976; Post-Beittenmiller, 1996). In 6 TLEs other leafy wax biomarkers (*n*-alkanes and *n*-alcohols) were undetectable (Fig. 4-5). Three extracts contained all 3 classes together, while the remainders yielded even carbon numbered *n*-alcohols but lacked *n*-alkanes. Of the remaining extracts 9 contained homologous series of LCFAs in low abundance (Fig. 4-5). The interpretation of such LCFA distributions is that they originated directly from carcass fats, incorporated *via* routing from the plants foraged by the animals (Halmemies-Beauchet-Filleau *et al.,* 2013; Whelton *et al.,* 2018). However, where

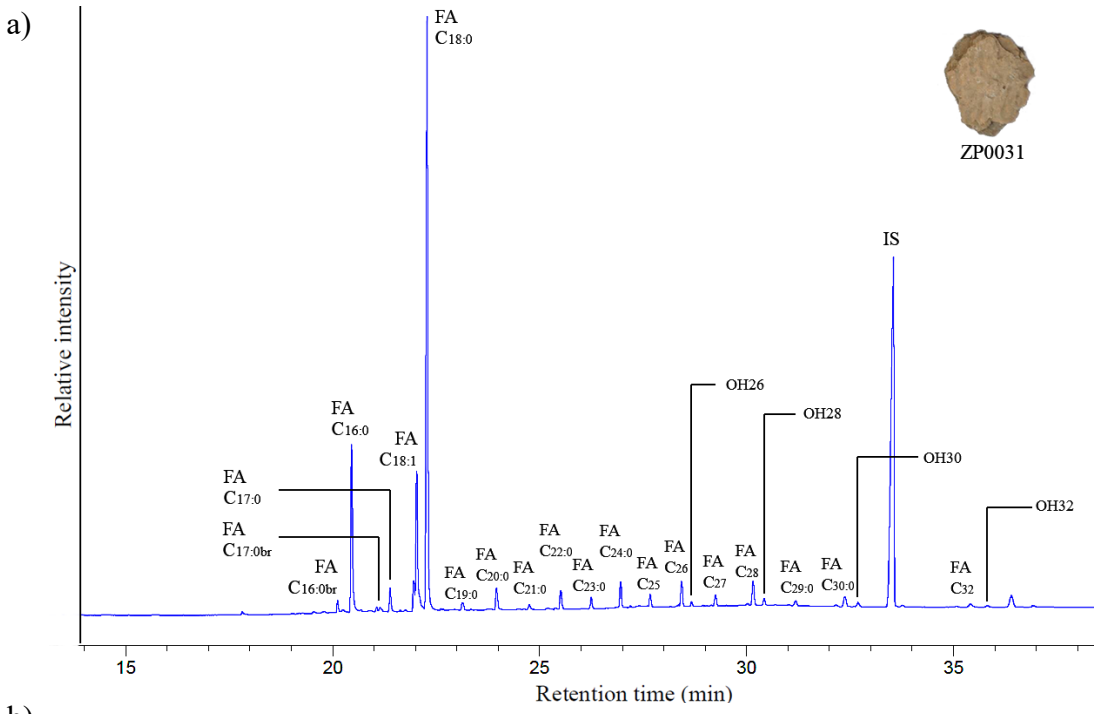
$\delta^{13}C$ values could be recorded, interestingly, they range from -32.6 to -26.8‰, indicating predominantly C_3 plant origins (Fig. 4-5). The remaining extracts yielding LCFAs will be discussed further in Section 4.3.1.1.

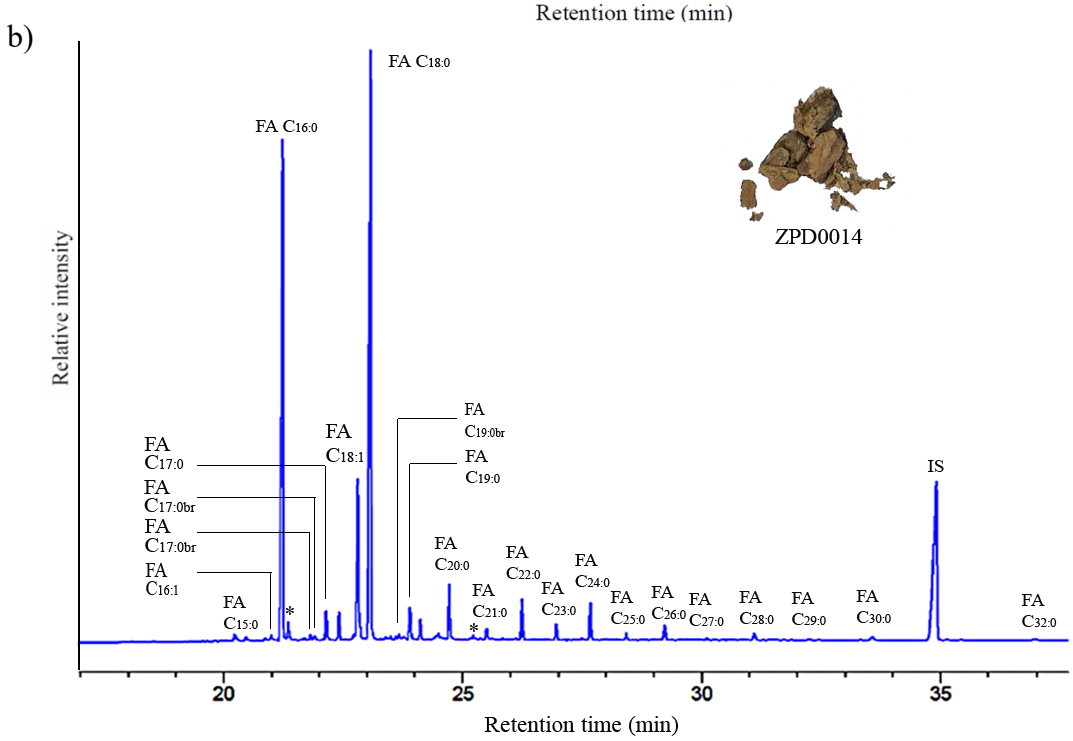
4.3 Investigating the exploitation of plant resources

Neolithic hunter-gatherers exploiting natural plant resources has long been regarded as a crucial food supplement worldwide. Apparently, the requirement of consuming and storing plant foods encouraged the invention of thermally resistant ceramic vessels as a human technological development (Jones, 2009). Processing raw plantstuffs within pottery vessels before consuming is required to render them edible, helping digestion and unlock their nutritional potential (Carmody and Wrangham, 2009).

It is clear now that the prehistoric dwellers of Zengpiyan did not develop any plant cultivations over the five thousand years of occupation of the cave, such that plant procurement relied on collecting wild plants (Chinese Academy of Social Science *et al.,* 2003). While no findings of archaeobotanical remains were recovered during the original Zengpiyan excavation in the 1970's, the re-excavation in 2001 involved soil flotation producing a variety of charred plant remains indicating the local diet was associated with early tuber starch and taro-based plant exploitation (Chinese Academy of Social Science *et al.,* 2003; Denham *et al.,* 2018).

Although plants would have been an important dietary food, unlike animal remains, the archaeological evidence of ancient people consuming plants is less readily identified, sometimes remaining invisible or amorphous. Before systematic flotation or quantitative analysis were applied in Chinese archaeological excavations, the study of recovered plant remains mainly focused on taxonomic identification based on morphology (Liu and Chen, 2012). Currently, the approaches to discover agricultural traces are based on pollen or starch analysis, microfossils, soil flotation (e.g. charred grains excavated from pits, hearths) or agricultural artefacts such as sickles.


a)
FA C18:0
ZP0031
IS
Relative intensity
FA C16:0
FA C18:1
OH26
OH28
OH30
OH32
FA C17:0
FA C17:0br
FA C16:0br
FA C19:0
FA C20:0
FA C21:0
FA C22:0
FA C23:0
FA C24:0
FA C25
FA C26
FA C27
FA C28
FA C29:0
FA C30:0
FA C32
15
20
25
30
35
Retention time (min)


b)
FA C18:0
FA C16:0
ZPD0014
Relative intensity
FA C19:0br
FA C19:0
FA C18:1
IS
FA C17:0
FA C17:0br
FA C17:0br
FA C16:1
FA C15:0
FA C20:0
FA C22:0
FA C24:0
FA C21:0
FA C23:0
FA C25:0
FA C26:0
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Retention time (min)

c)

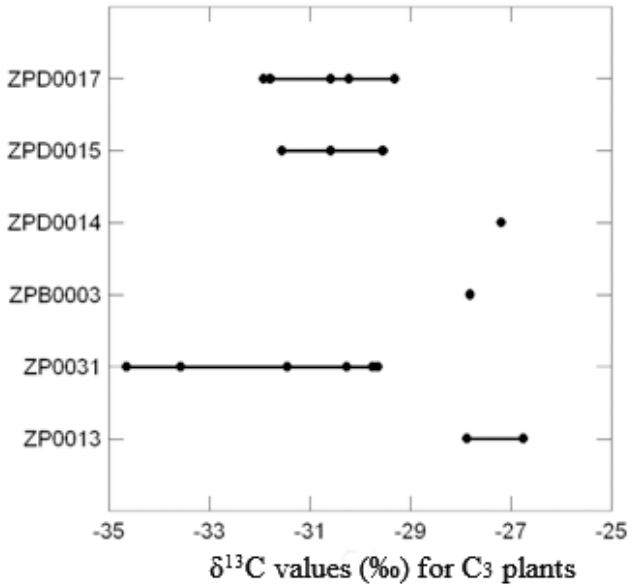


Figure 4-5 a) and b) Partial gas chromatograms of the TLE from ZPD0014 and ZP0031, showing the animal-derived $C_{16:0}$ and $C_{18:0}$ FAs together with an animal routed LCFA distribution. Key: FA Cx:y is the free fatty acid of carbon number X and Y degree of unsaturation, br comprises *iso*- or *anteiso*-branched fatty acid. OHx are *n*-alcohols with carbon number x. IS is the added internal standard (C_{34} *n*-alkane) and * denotes plasticiser contamination; c) Plot illustrating range of $\delta^{13}C$ values for the LCFAs derived *via* routing from the animal foraged vegetations. Each pot represented a LCFA in the extract. These isotope values plotted within the range of C_3 plants.

An alternative approach to the identification of plant processing within ancient pottery vessels can be based on detecting cuticular wax lipids in archaeological pottery, e.g. LCFAs, *n*-alcohols, *n*-alkanes, terpenoids or sterols (Evershed, 1993; Evershed, 2008). Lipid biomarker evidence of plant processing in pottery has been detected from archaeological sites in Britain, and Africa (Evershed *et al.,* 1991b; Cramp *et al.,* 2011; Dunne *et al.,* 2017, 2018; Hammann and Cramp, 2018). The earliest plant processing revealed within ceramic vessels come from Saharan pottery dating to from the Early to Middle Holocene in the Libyan Sahara (Dunne *et al.,* 2017).

At Zengpiyan almost half of the TLEs ($n = 9$; 47% of the potsherds containing residues) yielded plant biomarkers (*n*-alkanes and *n*-alcohols) or plant-derived LCFAs, indicative of plant processing in the vessels, albeit in trace amounts in some cases (Fig. 4-5). In most extracts, fatty acid distributions of plant origins are indicated by exhibited a high abundance of *n*-$C_{16:0}$ compared with *n*-$C_{18:0}$, typically a P/S ratio value ≥1.3 (Mills and White, 1994). However, it is not possible to assign a precise plant origin on the basis of fatty acids alone (Dunne *et al.,* 2017). The low abundance of plant-derived lipids is a consequence of the signal of plant-derived lipids being overlain by fat-rich animal products during the vessel use, as animal products produce much higher concentrations of lipids compared to plant (Charters, 1996; Evershed *et al.,* 1999). Furthermore, only a few species of plant foodstuffs have been shown to produce species-specific biomarkers, e.g. maize kernel yields C_{32} *n*-alcohol, with exhibiting a lighter $\delta^{13}C$ value,

or *Brassica* (cabbage) leaf wax components comprising mainly C_{29} *n*-alkanes (nonacosane), nonacosan-15-one and nonacosan-15-ol (Charters *et al.,* 1997; Reber *et al.,* 2004a).

4.3.1 Plant biomarkers: long-chain fatty acids (LCFAs), *n*-alcohols and *n*-alkanes

4.3.1.1 LCFAs

Previous organic residue analyses of archaeological potsherds have revealed $C_{16:0}$ and $C_{18:0}$ fatty acids are commonplace and dominate TLEs. LCFAs have only ever been seen in lower abundance than $C_{16:0}$ and $C_{18:0}$ fatty acids, the latter believed to derive from animal resources (Evershed *et al.,* 1997a). However, the proportions of LCFAs in 7 extracts were much higher than or equal in abundance to $C_{16:0}$ and $C_{18:0}$ fatty acids, with their distributions mostly maximizing at $C_{26:0}$ and $C_{28:0}$. The high abundance of LCFAs is extremely unusual although their concentrations are low (maximum 0.13 mg g^{-1}), while the concentrations of *n*-alkanes and *n*-alcohols were lower, or these components were absent. The presence of LCFAs up to *n*-C_{34} is strongly indicative of an origin in leaf or stem epicuticular waxes, although interestingly they are also a component of the root and bark polymer suberin, lignin, tannins or flavonoids (Walton, 1990).

Although LCFAs are known to derive from the ruminant dietary plants (Halmemies-Beauchet-Filleau *et al.,* 2013, 2014; Whelton, *et al.,* 2018; Section 4.2.1), the high abundance of LCFAs would be more diagnostic of direct processing of plant products in pottery. The carbon isotope values further confirmed these LCFAs as likely to originate from C_3 plants (Fig. 4-6).

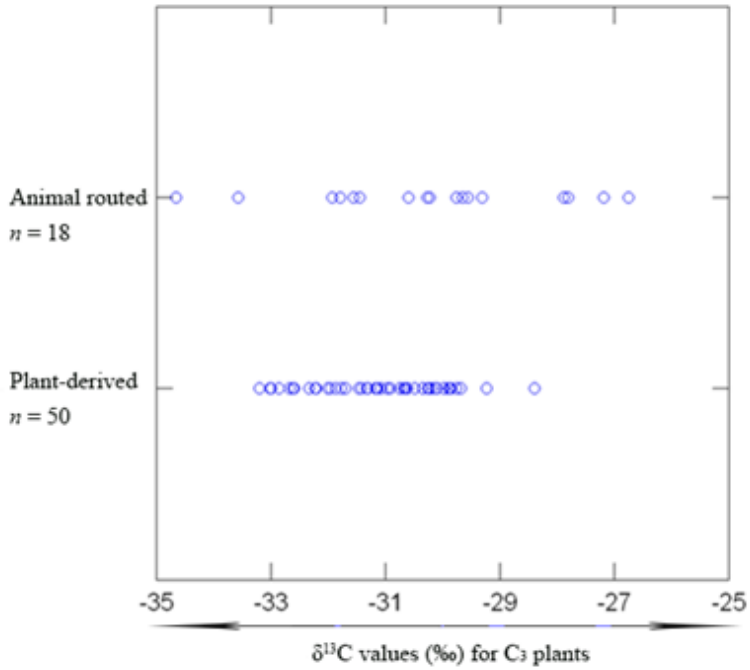


Figure 4-6 Plot showing the comparison of the range of $\delta^{13}C$ values for LCFAs in different types of residues preserved in pottery from Zengpiyan. Each point represents one LCFA. "Animal routed" refers to LCFA occurring within animal fat residues as a result of dietary assimilation into animal tissues, and "Plant-derived" LCFAs occurring in organic residues from direct plant processing.

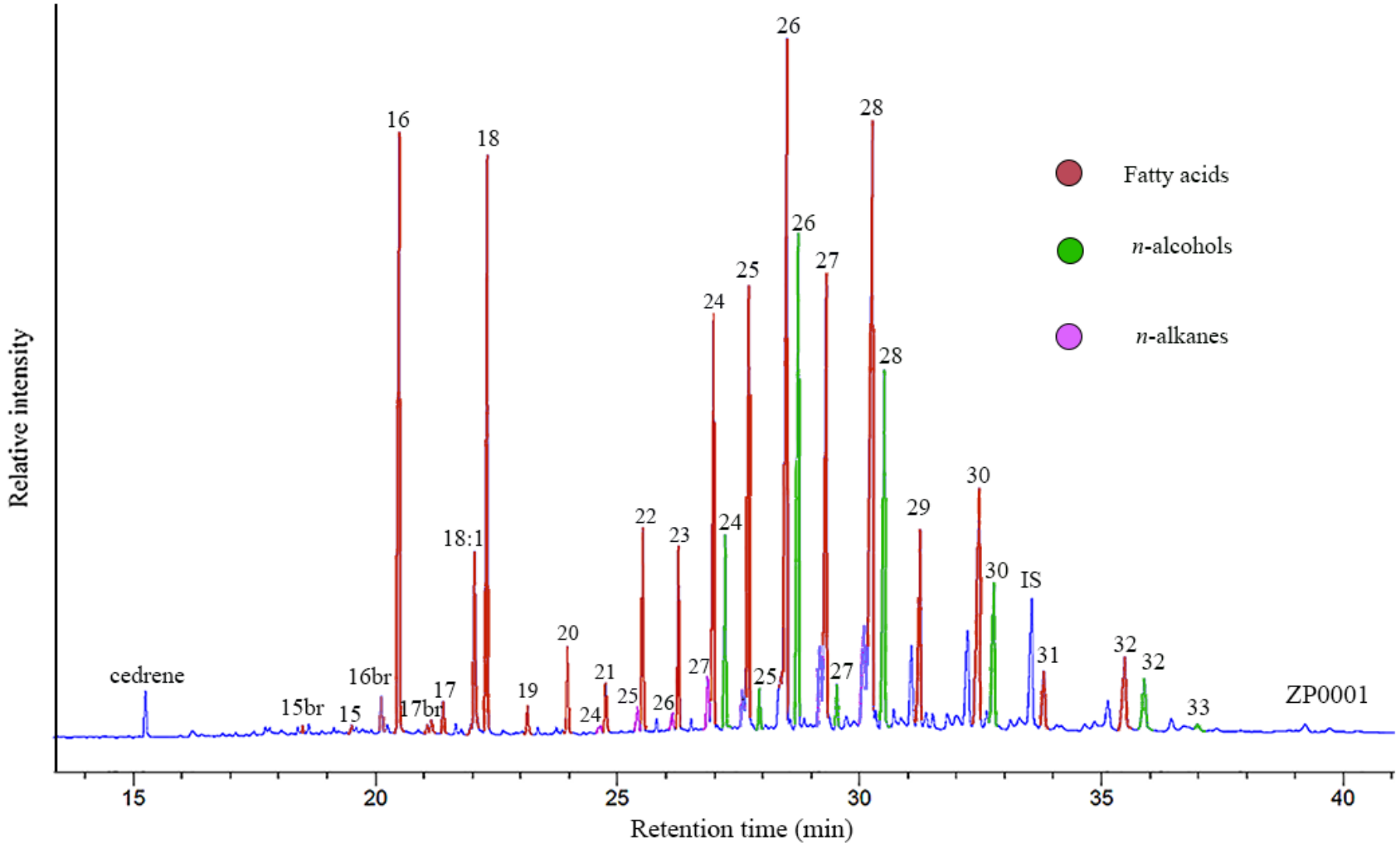
All the $\delta^{13}C$ values of long-chain fatty acids corresponded a C_3 plant origin (from -34.7 to -26.8‰). Interestingly, the ranges of carbon isotope values of long-chain fatty acids associated with animal carcass fats exhibited a broader range of isotope values than those deemed to be directly plant-derived. The unusually high abundances of LCFAs observed in the animal fat residues may reflect their enhanced resistance to leaching and degradation, rather than the mixed processing of plant and animal products in the same vessel.

Four lipid residues (ZP0001, 10, 11 and 26) yielded LCFAs, together with other plant biomarkers (*n*-alcohols and *n*-alkanes). To investigate ZP0001, 10 and 11 residues (ZP0026 yielded unusual high abundance of $C_{18:1}$ fatty acid, and will be analysed by other ways) further, they were re-analysed by chloroform/methanol extraction and HTGC and HTGC-MS (Correa-Ascencio and Evershed, 2014). The trimethylsilylated TLEs obtained from this method only contained a series of *n*-alcohols with a very low abundance of *n*-alkanes, with fatty acids being absent (Fig. 4-7). The absence of fatty acids in chloroform-methanol extract but presence in the acidified methanol extraction can be interfered to be related to the presence of fatty acid in the Zengpiyan as alkaline inorganic metal salts (e.g. Ca^{2+}, Mg^{2+}) on the potsherd surface or within the clay matrix. Such salts are poorly soluble in non-protic solvents (chloroform/methanol) and thus would not be extracted. However, the salts are broken down

in the acid methanol extraction, hence, their extensive appearance in the HTGC and HTGC-MS analyses. *n*-Alcohols and *n*-alkanes will not form such salt and thus are, extracted by the chloroform-methanol (Correa-Ascencio and Evershed, 2014). These inorganic metal ions potentially arise from the water used for cooking or the inclusions, e.g. crushed calcite was used as the major tempering agent in Zengpiyan pottery manufacture from Phase II. Calcite is a mineral composed of calcium carbonate ($CaCO_3$), providing Ca^{2+} ions for FA salt formation (Chinese Academy of Social Science *et al.,* 2003).

It is unknown why the internal standard does not appear in the gas chromatograms of the TLEs. Thus, the lipid concentrations of chloroform /methanol extraction cannot be calculated. Unfortunately, the trimethylsilylated TLEs had to be kept in a fridge for 2 days due to a delay in instrument availability, thus, the DAGs/MAGs peaks seen in GC could not be confirmed by GC-MS because of degradation of their TMS derivative.

a)


Relative intensity
Retention time (min)
15
20
25
30
35
40
Fatty acids
n-alcohols
n-alkanes
cedrene
15br
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16br
16
17br
17
18:1
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b)

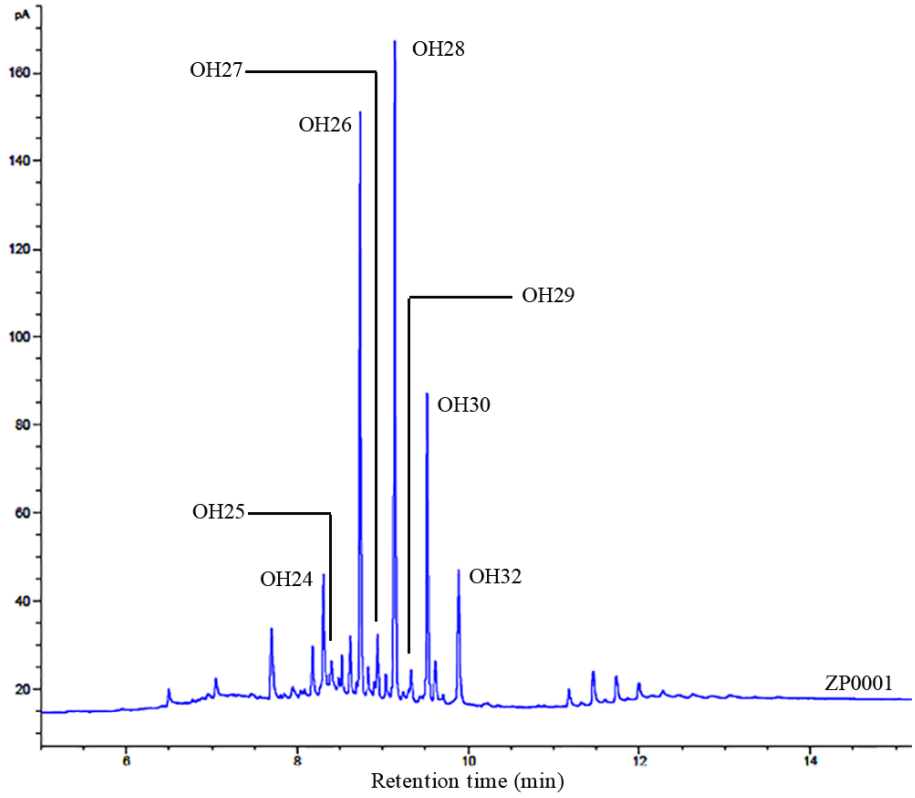

Figure 4-7 a) Partial gas chromatograms of the acidified methanol extracted lipids from ZP0001; b) Partial high-temperature gas chromatogram of the ZP0001 TLE. Key: red peaks denote the free fatty acids of X carbon number, dark green peaks represent *n*-alcohols of X carbon number, purple peaks are *n*-alkanes of X carbon number, 17br comprises *iso*- and *anteiso*-branched fatty acids containing 17 carbon atoms and $C_{X:Y}$ are unsaturated fatty acids of carbon number X and Y degree of unsaturation. OH_X are *n*-alcohols of carbon number X. IS is the added internal standard (C_{34} *n*-alkane).

The residues recovered ZP0010 contained the unsaturated FA $C_{18:1}$ and $C_{18:2}$, which are indicators of plant oils, however, unsaturated fatty acids normally not survive over such long timescales and their presence should be viewed with caution (Fig. 4-8; Copley *et al.,* 2001). The $\delta^{13}C$ values of the *n*-$C_{16:0}$ and *n*-$C_{18:0}$ fatty acids of ZP0010 were also unusual since they suggested a mixture of ruminant dairy and adipose fats, due to less depleted $\delta^{13}C$ values of *n*-$C_{18:0}$ fatty acid relative to *n*-$C_{16:0}$, -23.6 and -20.2‰, respectively. The less depleted $\delta^{13}C$ values suggest a C_4 origin. Interestingly, the $\delta^{13}C$ values of its LCFAs co-occurring in the same extract ranged from –33.2 to –30.6‰ and the even-numbered *n*-alcohols ranged from -32.2 to -30.1‰, respectively, which indicates an origin from terrestrial C_3 plants. No useful information was obtained from the HT-GC analysis of the chloroform/methanol extract of ZP0010, with only a low concentration of *n*-alcohols and high abundance of plasticiser detected.

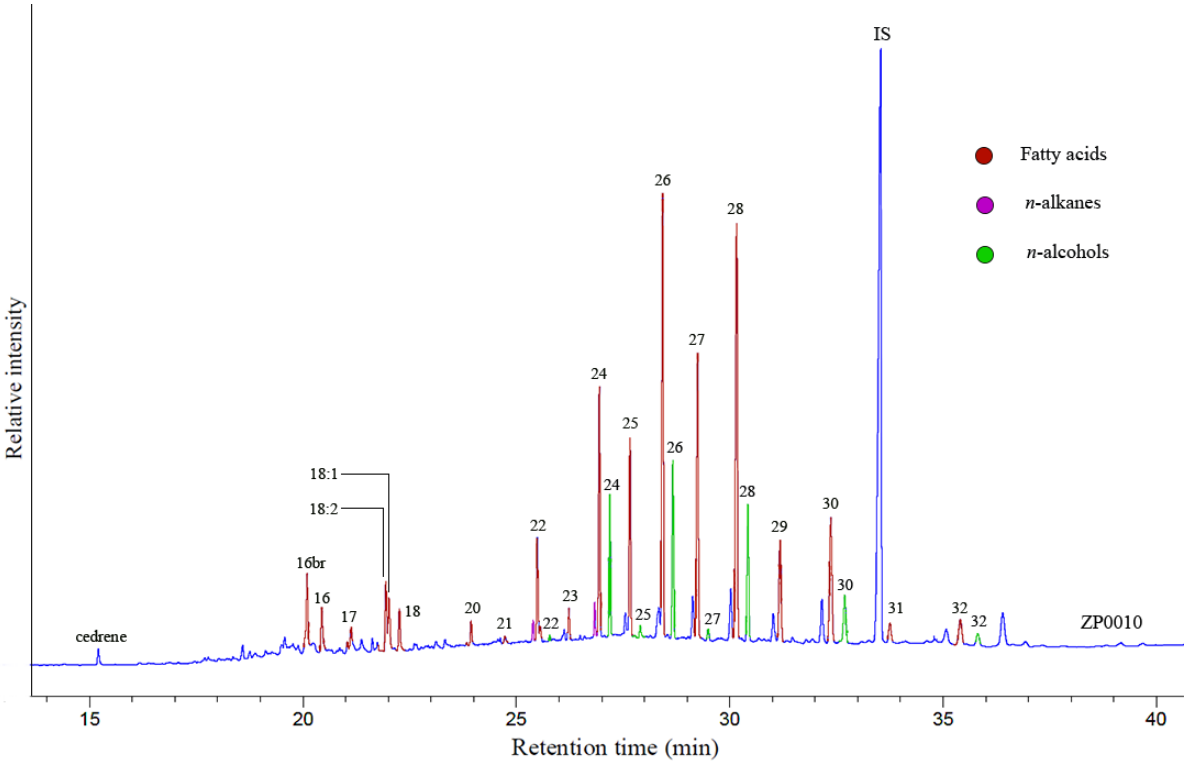

Figure 4-8 Partial gas chromatograms of the acidified methanol extracted lipids from ZP0010. Key: red peaks denote free fatty acids in X carbon number, dark green peaks represent *n*-alcohols in X carbon number, purple peaks are *n*-alkanes in X carbon-numbered, 17br comprises iso- and anteiso-branched fatty acids containing 17 carbon atoms and $C_{X:Y}$ are unsaturated fatty acids with carbon number X and Y degree of unsaturation. IS is the added internal standard (C_{34} *n*-alkane) and * denotes plasticiser contamination.

To further confirm lipid origin the $\Delta^{13}C$ values of the fatty acids $C_{16:0}$ and $C_{18:0}$ were determined (Fig. 4-9). One residue containing plant-produced LCFAs plots in the non-ruminant adipose range, two in the ruminant adipose fats range, and one at the ruminant adipose/dairy fat boundary but must be an ruminant adipose fat outlier since dairy fats could not have feature in the period of occupation of Zengpiyan (Chinese Academy of Social Science *et al.,* 2003).

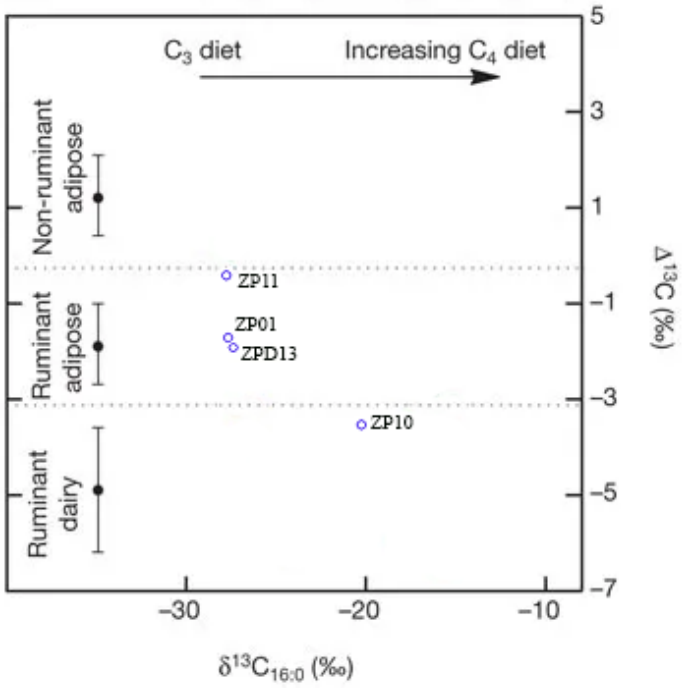

Figure 4-9 Plot of $\Delta^{13}C$ ($\delta^{13}C_{18:0}$ - $\delta^{13}C_{16:0}$) values plotted against $\delta^{13}C_{16:0}$. The $\delta^{13}C$ values of modern reference fats were adjusted for post-Industrial Revolution effects of fossil fuel burning by the addition of 1.2‰ (Friedl *et al.,* 1986). The confidence ellipses (±1 σ) represent the $\delta^{13}C$ values of modern reference animals in Britain (raised in a pure C_3 diet), Africa, Kazakhstan, Switzerland and the Near East (Copley *et al.,* 2003; Dunne *et al.,* 2012).

4.3.1.2 *n*-alkanes and *n*-alcohols

Of the seven extracts containing *n*-alcohols only 3 yielded *n*-alkanes which had CPI values of 2.90, 6.3 and 49.28, inferring that the *n*-alkanes present derived from epicuticular waxes of higher plants (Tulloch, 1976). The $\delta^{13}C$ values *n*-alkanes present in the extract of ZP0026 indicate a C_3 plant origin (Fig. 4-10), the similarity of the values for the two major homologues suggest a common origin.

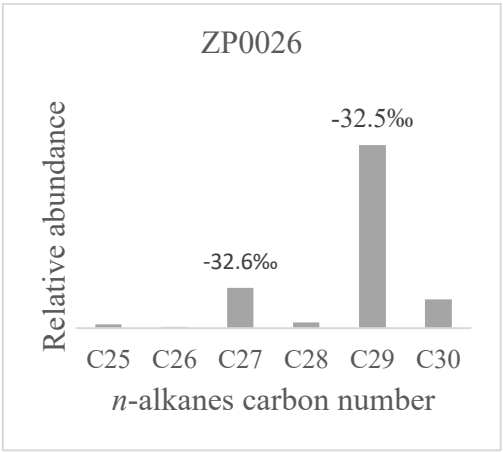

Figure 4-10 Histogram showing the distributions of plant-derived *n*-alkanes in ZP0026. Cx refers to *n*-alkanes of carbon number x. Numbers on top of the bars indicate the $\delta^{13}C$ value of each *n*-alkane.

Mostly, *n*-alkanes occur in low abundance in the lipid extracts. This contrasts with the fact that *n*-alkanes are often the major components of plant leaf waxes (Koch and Ensikat, 2008), occasionally being the dominant plant lipids of organic residues in pottery (Evershed *et al.,* 1991b; Charter *et al.,* 1997; Cramp *et al.,* 2011). However, in some cases, *n*-alkanes did not survive, e.g. the lipids extracted from the Takarkori or Uan Afuda potsherds (Dunne *et al.,* 2017). Alternatively, the plants processed within pottery vessels would be expected to be incapable of producing *n*-alkanes. It has been found that many of gymnosperm plants (i.e. conifers, *Ginkgo* and cycads) contain no trace of any *n*-alkanes (Diefendorf *et al.*, 2011). The archaeobotanical assemblage in Zengpiyan includes *Pinus massoniana*, whose seeds are edible (Chinese Academy of Social Science *et al.,* 2003). Nineteen of the TLEs contained the diterpeniods, dehydroabietic, pimaric, isopimaric and 7-oxodehydoabietic acids, inferring the exploitation of *Pine spp.* Hence, it can legitimately argue that gymnosperm seeds were processed in pottery. The occurrence of diterpenoids in pottery is discussed further below.

In the organic residues, long-chain *n*-alcohols can be the products of wax ester hydrolysis, and normally appear as C_{22} to C_{34} homologues (Chibnall *et al.,* 1934; Eglinton and Hamilton, 1967). Among 8 lipid extracts yielding *n*-alcohols, 3 yielded minor traces of *n*-alcohols (Fig. 4-11), accompanied by LCFAs possibly arising *via* routing from the animal's diets. The *n*-alcohols in the remaining lipid residues exhibited a relative higher abundance, along with the presence of plant-derived LCFAs, also *n*-alkanes, possibly the residues of plant processing in vessels (Fig. 4-11).

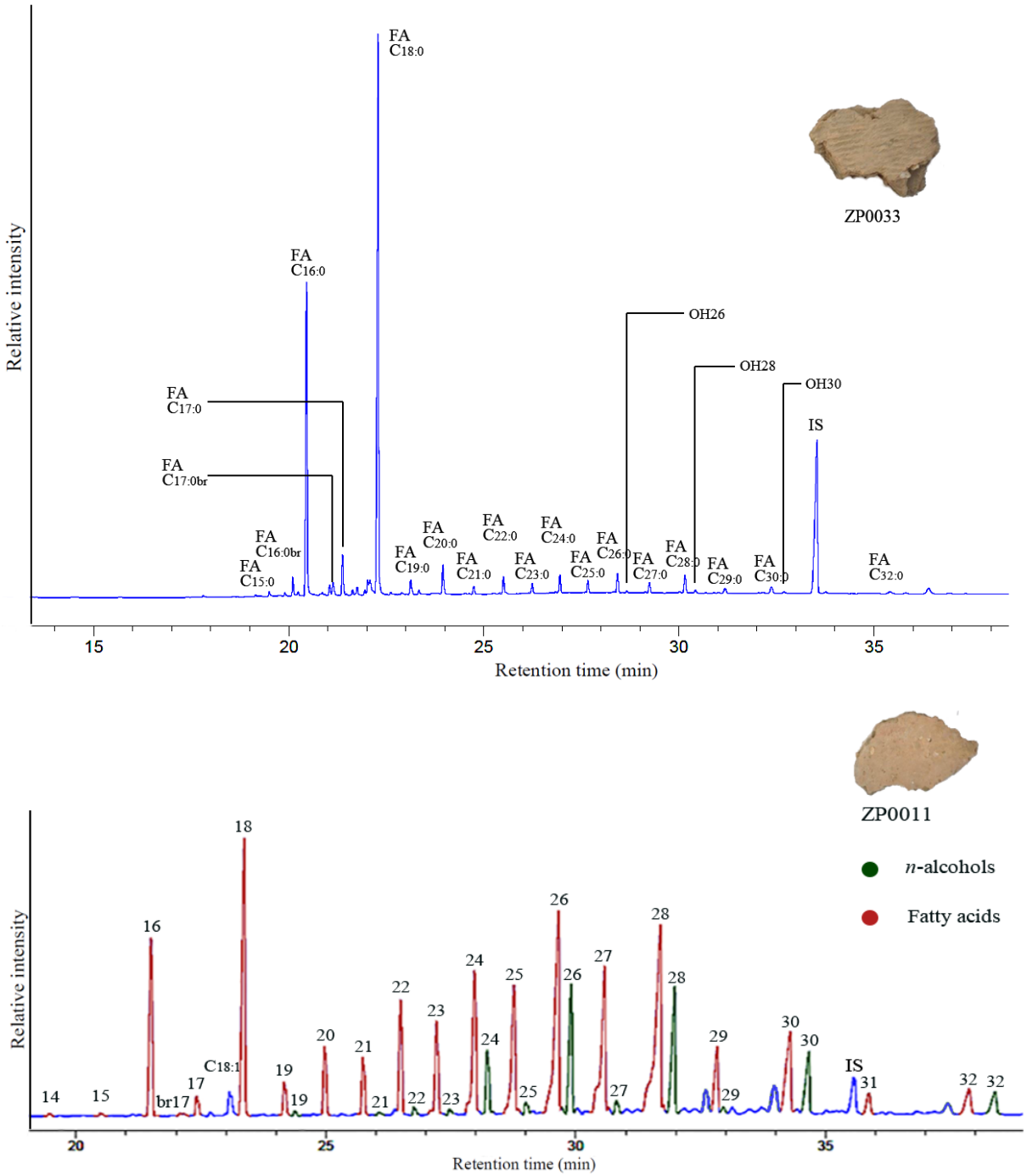

Figure 4-11 Partial gas chromatograms of the TLEs from ZP0033 and ZP0011, illustrating two types (higher and lower abundance) of *n*-alcohol distributions. Key: red peaks denote the free fatty acids in X carbon number, dark green peaks represent *n*-alcohols in X carbon number, 17br comprises *iso*- and *anteiso*-branched fatty acids containing 17 carbon atoms and $C_{X:Y}$ are unsaturated fatty acids with carbon number X and Y degree of unsaturation. OH_X are *n*-alcohols with carbon number X. IS is the added internal standard (C_{34} *n*-alkane).

4.3.2 Diterpenoids

Diterpenoids, dehydroabietic, pimaric, isopimaric and 7-oxodehydoabietic acids were detected in 16 extracts as TMS esters, suggesting the exploitation of *Pinus* sp. (Pollard and Heron, 2008; Reber and Hart, 2008). As to why the methyl ester were not formed during the acidified methanol is not completely clear but may be due to the hindered nature of the carboxyl group.

An example gas chromatogram of a lipid extract containing diterpenoids is shown in Figure 4-12. The resin of *Pinus* sp. is one of the most widespread diterpenoid resins seen in the archaeological record. The use is widespread as a waterproofing agent, adhesives and in mummy balms (the mass spectra of the characteristic diterpeoids are shown in Fig. 4-13; Charters *et al.,* 1993; Buckley and Evershed, 2001; Buckley *et al.,* 2004; Colombini *et al.,* 2005; Pollard and Heron, 2008). Chemical alterations to diterpenoids are caused by oxidation and reduction reactions due to environmental factors or aging, e.g. dehydroabietic acid exposed to oxygen oxidatively degrades to 7-oxodehydroabietic acid in aged coniferous resins (Mills and White, 1994; Pollard and Heron, 2008).

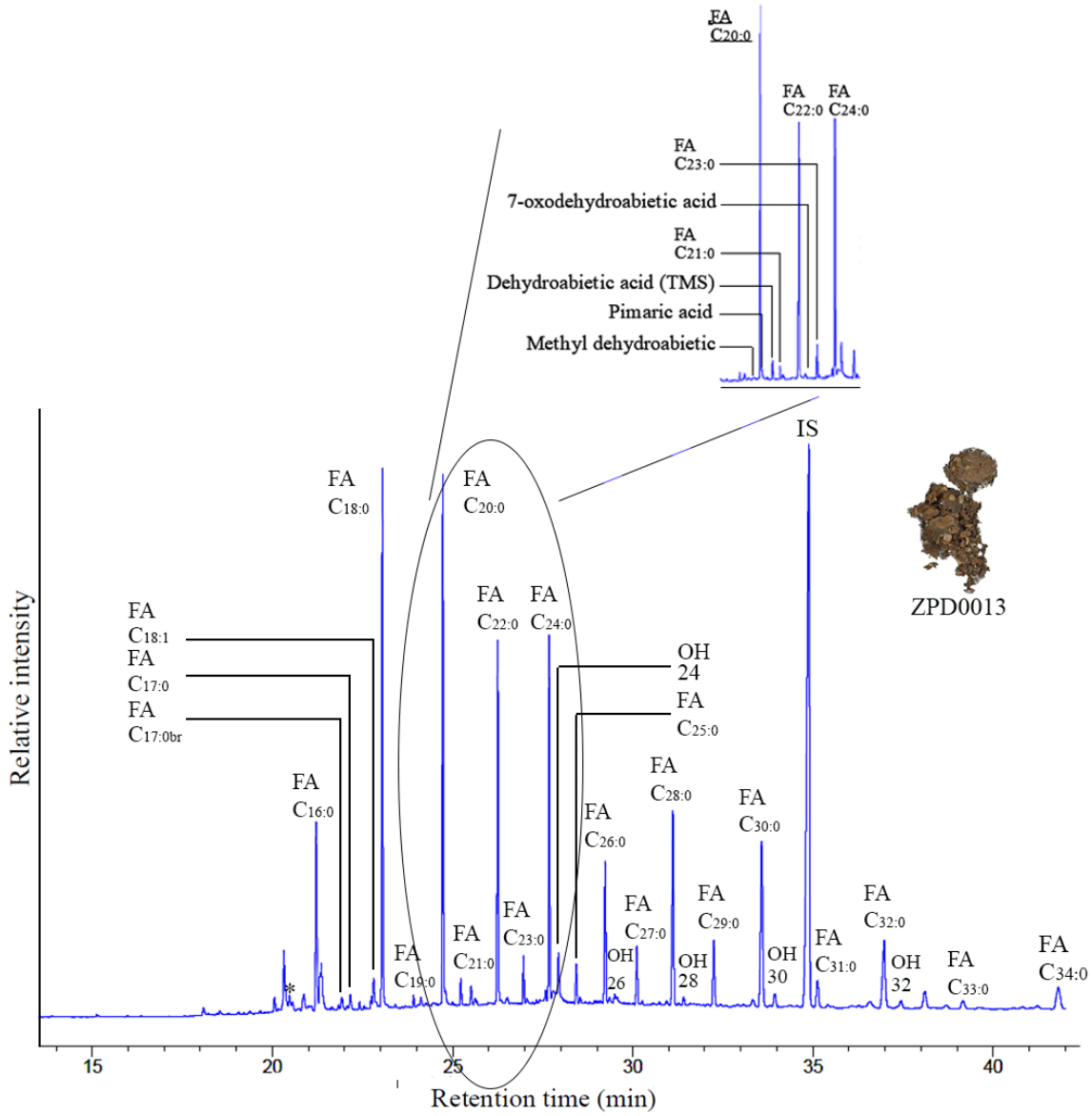

Figure 4-12 Partial gas chromatogram of the TLEs from ZPD0013, illustrating the distribution of diterpenoids characteristic of pine resin or pitch. Key: FA Cx:y are the free fatty acid of carbon number X and Y degrees of unsaturation, br comprises *iso*- or *anteiso*-branched fatty acid. IS is the added internal standard (C_{34} *n*-alkane) and * denotes plasticiser contamination.

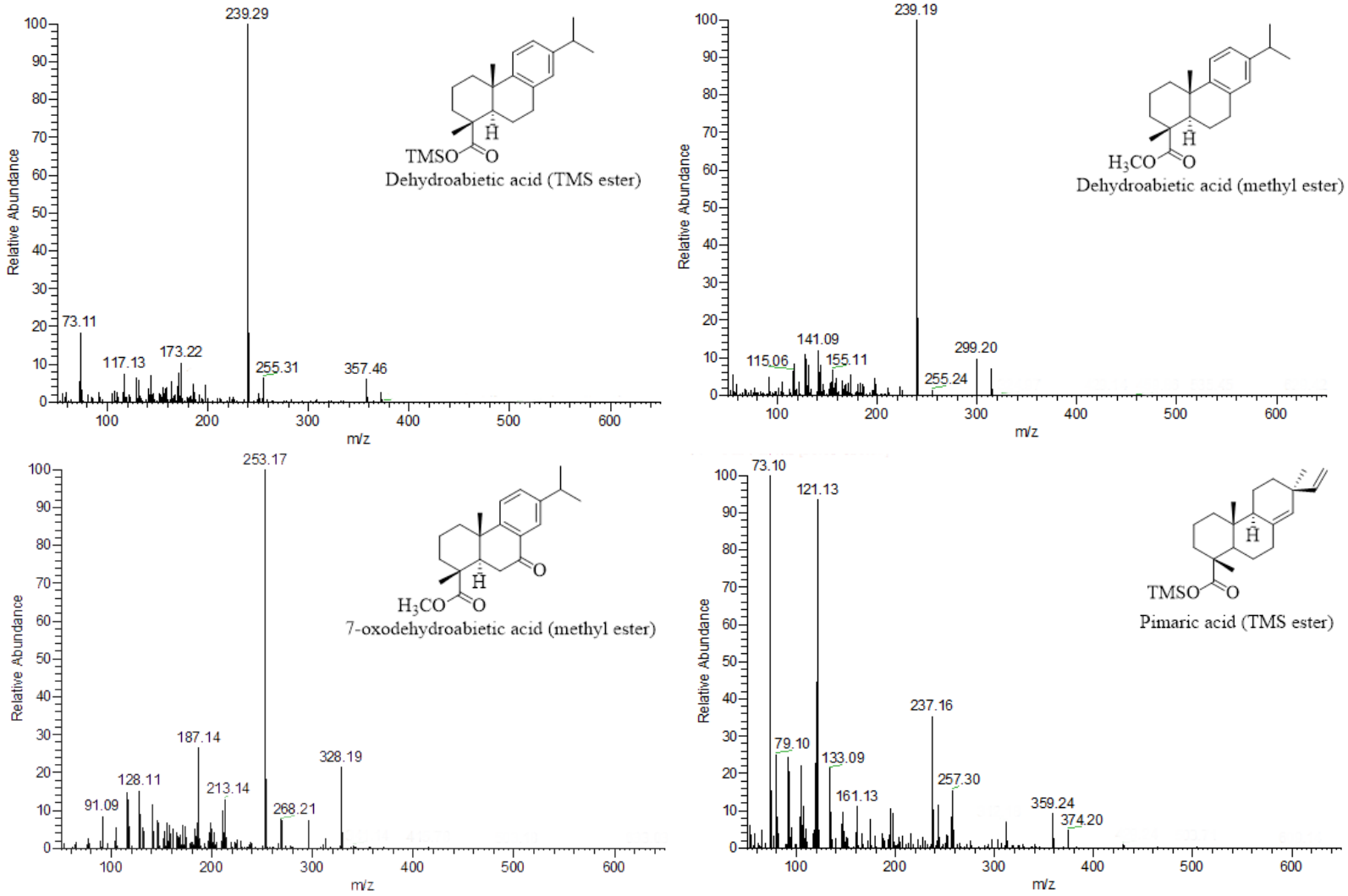

Figure 4-13 Mass spectra of diterpenoids dehydroabietic acid (TMS ester and methyl ester), 7-oxodehydroabietic acid (methyl ester), and pimaric acid (TMS ester) identified in extracts from Zengpiyan.

Looking at the chemistry of the diterpenoids in the extracts in more detail, methyl dehydroabitate was present in 8 TLEs co-occurring with dehydroabietic TMS ester, with the abundance of the methyl ester lower than TMS ester. The abundance of dehydroabietic methyl ester (and retene) is regarded as an indicator of aged pine pitch obtained from heat treatment of resinous wood or resin pyrolyzing and producing methanol (Evershed *et al.,* 1985; Mitkidou *et al.,* 2008), while tars produced by heating resins alone comprise resin acids but no methyl esters. However, given the derivatization method was methylation, it is not possible to distinguish whether the methylated diterpenoid acids came from a tar/pitch or were derivatization products (Colombini *et al.,* 2005; Pollard and Heron, 2008). Only three potsherds were submitted to solvent extraction, and methyl dehydroabietic acid was absent from the extracts. Given no retene was detected with methyl dehydroabitate indications are that the diterpenoids do not derive from pine pitch.

Diterpenoids are one of the most widely used archaeological biomarkers for higher plant exploitation in archaeology, due to their specific origin in pinus species (Hayek *et al*., 1990; Charters *et al.,* 1993; Mills and White, 1994). The occurrence in the extracts is consistent with the fact that during the Neolithic, the Zengpiyan hinterlands would have been covered by a subtropical evergreen broad-leaved forest comprising resin-producing trees, notably *pine* and *palm* sp.. Only one indigenous *Pinus* sp. exists in this area, *Pinus massoniana* Lamb., which probably provided resin (Chinese Academy of Social Science *et al.,* 2003). Evidence of early resin exploitation in China has been discovered in the form of a black adhesive substance on microlithic tools likely used as a glue to fasten inlaid microliths at Yuanyangchi, a Late Neolithic site in Gansu (Archaeological Team and Archaeological Survey Team, 1982).

An archaeological study of Zengpiyan pottery, indicated potters potentially utilized pine resin during the pottery manufacture to increase the viscosity between tempering agents and slabs, however, firing would destroy the resin. However, a recent experimental study has suggested that the presence of trace levels of diterpenoid in pottery should be interpreted with caution as these can be residues of the wood condensate derived from the firing of the pottery (Reber *et al.,* 2019).

Interestingly, the sesquiterterpene, α-cedrene, was present in 3 potsherds (ZP0001, 10, 11), along with a series of LCFAs, *n*-alkanes and corresponding to complex lipid distributions (e.g. Fig. 4-7, 8, and 11). As the previously published herb contexts of Zengpiyan, cedrane (mass spectra are shown in Appendix 2) possibly originated from specific tree resins [e.g. China fir,

Cunninghamia lanceolate (Lamb.) Hook; *Cupressus funebris*], the Chrysanthemum family flower, or an aromatic herb, specifically *Artemisia annua* L. (sweet wormwood; Compositae) or *A. argyi* in the wormwood/mugwort genus (Chinese Academy of Social Science *et al.,* 2003; McGovern *et al*., 2014).

In summary, the presence of these diterpenoids in the TLEs suggests the exploitation of an expanded range plant resources were processed within pottery vessels at Zengpiyan. However, it is not possible to deduce the functional use of the resins. Structural alterations are evident which likely occurred due to pyrolysis or oxidation during burial (Mill and White, 1994; Pollard and Heron, 2008). The presence of diterpenoid resin components could result from waterproofing or adhesive (Mitkidou *et al.,* 2008), or be the absorbed residues from processing pine resin (Reber and Hart, 2008), or left as a residue from fuel (Whelton, 2016; Reber *et al.,* 2019).

4.4 Benzoic acid derivatives

Although normally present in very low abundance in nature, TMS derivatives of benzoic acid were detected in 9 extracts, with two of them containing 2-methyl benzoic acid (mass spectra are shown in Appendix 2). These carboxylic acids occur naturally in many balsamic resins (e.g. *Myroxylon*, leguminous trees that yield Peru balsam, and *Xanthorrhoea,* the Australian grass tree), also associated with suberin, lignin and flavonoids (Kolattukudy, 1980; Langenheim, 2003). Two extracts (ZP0001, 10) yielded 2-methyl benzoic acid together with a homologous series of LCFAs, *n*-alkanes, and *n*-alcohols, which are all plant components, suggesting a plant origin. The use of these low molecular weight compounds as archaeological biomarkers requires careful consideration as they are volatile and could be contaminants.

4.5 The exploitation of aquatic commodities

Located within the proximity to several streams and the Li River, the potential existed for the inhabitants of Zengpiyan to gather freshwater aquatic resources. This is suggested by the substantial quantity of shellfish shells and bone tools (fishing spears) recovered, pointing to extensive freshwater fish consumption. In addition to fish remains, cooking experiments replicating the use of the earliest pottery originates confirmed the consumption of freshwater shellfish would have required heating process within pottery vessels, suggesting that the pottery produced at least in the early phase at Zengpiyan period was associated with processing aquatic resources (Chinese Academy of Social Science *et al.,* 2003).

Twelve of the TLEs (92%) contained 9,10-dihydroxystearic acid, and it was the only vicinal dihydroxy fatty acid detected in all TLEs. Although this is indicative of double-bond position in the precursor unsaturated fatty acid (Fig. 4-14), the source of precursor unsaturated fatty acid is unknown. Only one extract, ZP0033, contained thermally produced C_{18} APAAs although there were no charred remains on the surface (Fig. 4-14). No C_{20} or C_{22} APAAs were present. Isoprenoid fatty acids, pristanic and phytanic acids were detected in ZPB0003. Since the C_{18} DHYA, C_{18} APAAs and phytanic acid can be found in terrestrial organisms, these biomarkers are not characteristic enough to conclude that aquatic products were processed within pottery vessels (Evershed *et al.,* 2008a; Cramp and Evershed, 2014; Lucquin *et al.,* 2016a; Casanova, 2018).

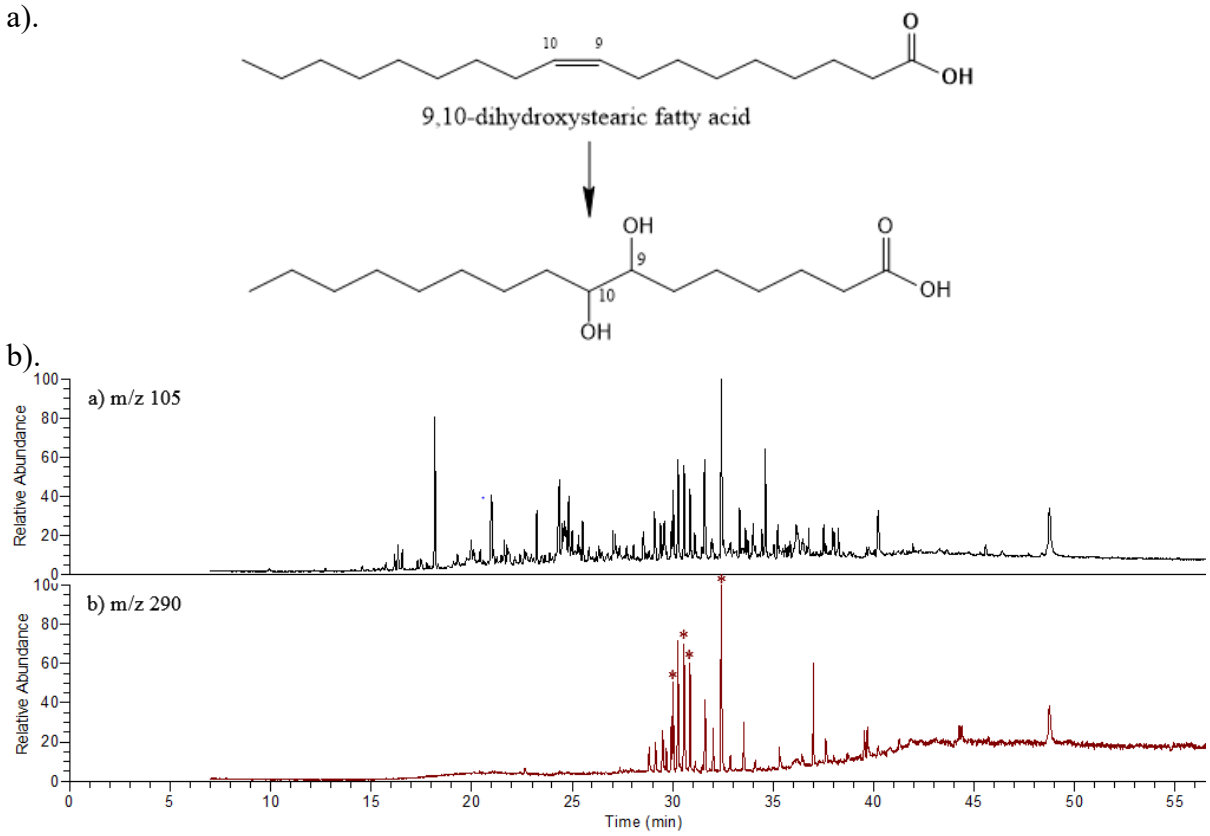

Figure 4-14 a). Formation of 9,10-dihydroxystearic fatty acid; mass chromatograms of a) base peak, *m/z* 105, b) molecular ion, *m/z* 290 of C_{18} APAA methyl esters from ZP0033, * illustrates the isomer distribution of C_{18} APAAs.

Subtle discriminations between freshwater and other resources because the carbon source of freshwater comes from various origins. Lipids of marine origin yield less depleted carbon isotope values than those from freshwater organisms. Freshwater resources exhibit very variable isotopic compositions, correlating with the degradation of organic matter or dissolved

inorganic carbon. The reference $\delta^{13}C$ values of freshwater commodities can overlap ruminant and non-ruminant adipose fats, and even C_3 plants (Dufour *et al.,* 1999).

The carbon isotope proxy used below was based on reference lipid $\delta^{13}C$ values from modern and archaeological animal bones/tissues obtained from Britain and Japan (66.7% confidence; Fig. 4-15; Lucquin *et al.,* 2016b). Of 8 extracts which plotted in the freshwater ellipse, however, only ZP0033 yielded C_{18} APAAs. However, the formation of APAAs requires heating to ca. 300°C thus the absence of these biomarkers does not rule out a freshwater organism origin for these residues.

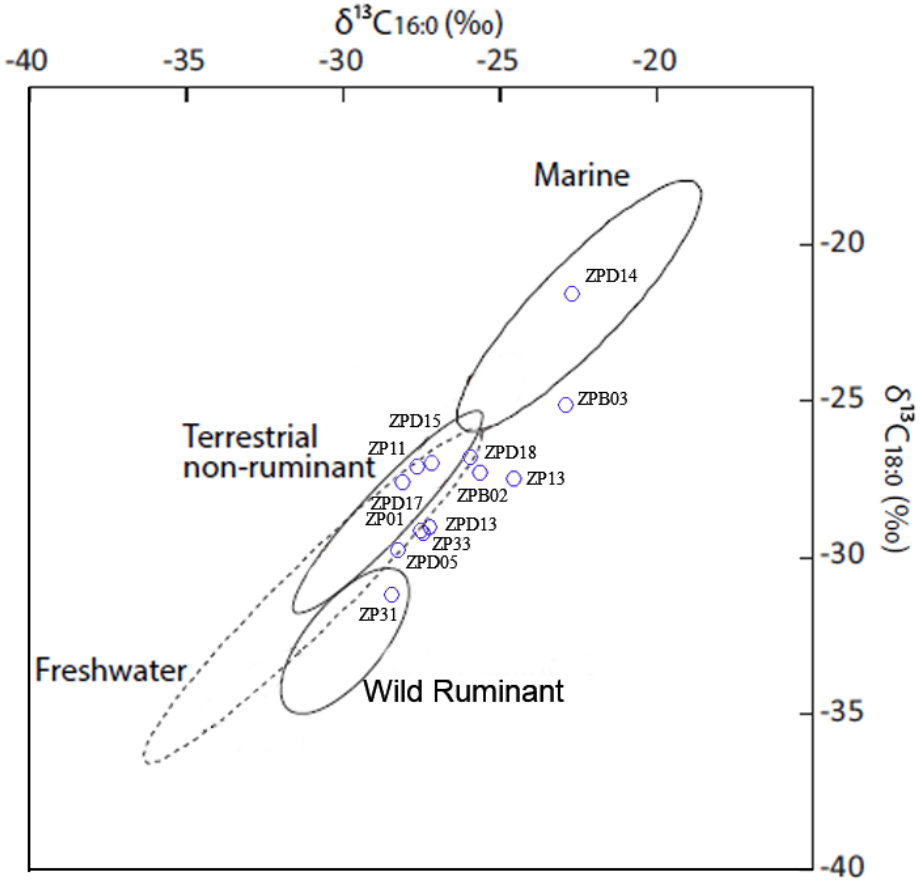

Figure 4-15 Scatter plot illustrating the $\delta^{13}C$ values of $C_{16:0}$ plotted against $C_{18:0}$ fatty acids from Zengpiyan extracts plotted within confidence ellipses from reference modern or archaeological bones/tissues from British and Japan (P=0.667; adapted from Lucquin *et al.,* 2016b).

Zengpiyan is situated on a limestone plain, boarded by streams and the Li river, far away from the coast. Due to ^{13}C-depleted inorganic carbon from limestone ($CaCO_3$) dissolving into streams, lipids from these freshwater species are anticipated to exhibit depleted $\delta^{13}C$ values, even less than terrestrial animals (Dufour *et al.,* 1999; Cuna *et al.,* 2001).

4.5 Other diagnostic compounds

Glycerol rarely survives in the archaeological seeds and bones, due to its highly water solubility. The survival of glycerol in 2 potsherds suggests the burial environment was very dry as this compound will completely leached from pottery vessels in waterlogged burial environments (Copley, 2002).

Cholesterol is the most abundant sterols in mammal tissues and a component of the skin surface. It was detected in 12 extracts. However, it is complicated to apply cholesterol as an archaeological biomarker due to its low abundance in the TLEs and its ubiquitous occurrence (e.g. as a potential contaminant arising from directly handling of artefacts; Evershed, 1993; Hammann and Cramp, 2018). Furthermore, cholesterol has been shown to be degraded when heated in the presence of high abundances of fatty acids making its occurrence in animal fat residues in pottery unpredictable and, hence, use as a biomarker of animal product processing unreliable (Hammann *et al.,* 2018). No other sterols were detected in any potsherds.

4.6 Unusual lipid distributions: unsaturated FA $C_{18:1}$

The mono-, di-, and triunsaturated C_{18} fatty acids are common in nature, with $C_{18:1}$ fatty acid being the most prevalent of fresh ruminant adipose tissues (McDonald, 2002). However, these unsaturated fatty acids have limited capacity to survive during long-term burial due to their susceptibility to oxidative degradation within archaeological pottery, hence they are normally absent or only minor components of lipid extracts (Copley, 2002). However, $C_{18:1}$ fatty acid (oleic acid) was a dominate component in 4 TLEs (ZP0020, ZP0026, ZP0028, ZP0032; Fig. 4-16), coupled with relatively high abundance of $C_{18:2}$, without any detection of its degradation products, e.g. dicarboxylic acids. As oleic acid is ubiquitous in nature, it is impossible to deduce its origin to specific plant oils or animal fats (Copley, 2002). Such lipid distributions have rarely been encountered in archaeological potsherds and are usually ascribed to contamination by post-excavation handling. Therefore, deeper surface cleaning was performed to decrease any surface or exterior contamination during the excavation (e.g. direct contact physical touching). Comparison of the previous gas chromatogram of ZP0020 with the deeper cleaned ones, showed an increased in the proportion of oleic acid from 23.8% to 34%, although less plasticizer contamination was observed (Fig. 4-16). Otherwise, the lipid profiles of two analyses were quite similar, without any change of the most abundant fatty acids.

The P/S values of these extracts varied from 1.39 to 2.12, although the $C_{16:0}$ and $C_{18:0}$ fatty acids were present in quite low abundance, inferring a plant origin (Mills and White, 1994). To better specify the origin of unsaturated FA $C_{18:1}$, these extracts were all submitted to GC-C-IRMS. The $\delta^{13}C_{18:1}$ values were less depleted, ranging from –19.8‰ to -15.6‰, indicating a C_4/marine origin. In terms of the major fatty acids, $C_{16:0}$ and $C_{18:0}$ in these extracts were too weak for their $\delta^{13}C$ values to be determined. Interestingly, the $\delta^{13}C$ values *of* *n*-C_{27} and *n*-C_{29} alkanes in ZP0026 II were -32.6‰ and -32.7‰, respectively, unambiguously assigning them to as of C_3 plants origin (Bi *et al.,* 2005).

The high abundance of $C_{18:1}$ in archaeological samples is unusual but has been observed at the Qasr Ibrim, Egypt, along with short-chain fatty acids in desiccated seeds. Such a distribution was inferred as arising due to the unusual level of protection conferred by the seeds when compared with archaeofaunal remains, preventing the oxidation during use and burial (Copley *et al.,* 2001). Processing seeds within pottery vessels is also exemplified by prehistoric people in Samburu, Kenya. The seeds from *Cyphostemma* sp. was boiled within pottery for obtaining soft and sticky substance, *lpulei*, *Balanites orbicularis* Sprague to extract their oil, or *sagaram*, *Acacia tortilis* (Forssk.) Hayne to release their nutrients into drinkable liquid (Grillo, 2014).

Together the variations in the lipid compositions from the two extractions and the property of unsaturated FA $C_{18:1}$, the lipid component originated from ancient subsistence can legitimately be considered as making sense. The cave is only one meter above the surrounding plain, which can be easily flooded. Percolating groundwater used to flood the exploratory shaft due to rapid fluctuation of water level during monsoon (Guo *et al.,* 2015). Notably, the high concentration of LCFAs and unsaturated FA $C_{18:1}$ in the residues suggests they have been kept under the enhanced protection to leaching and/or degradation. The cave afforded the favourable depositional environment, wetter condition, based on Zengpiyan geographical features, also, as the pottery, their matrices were possibly unique compared to other sites.

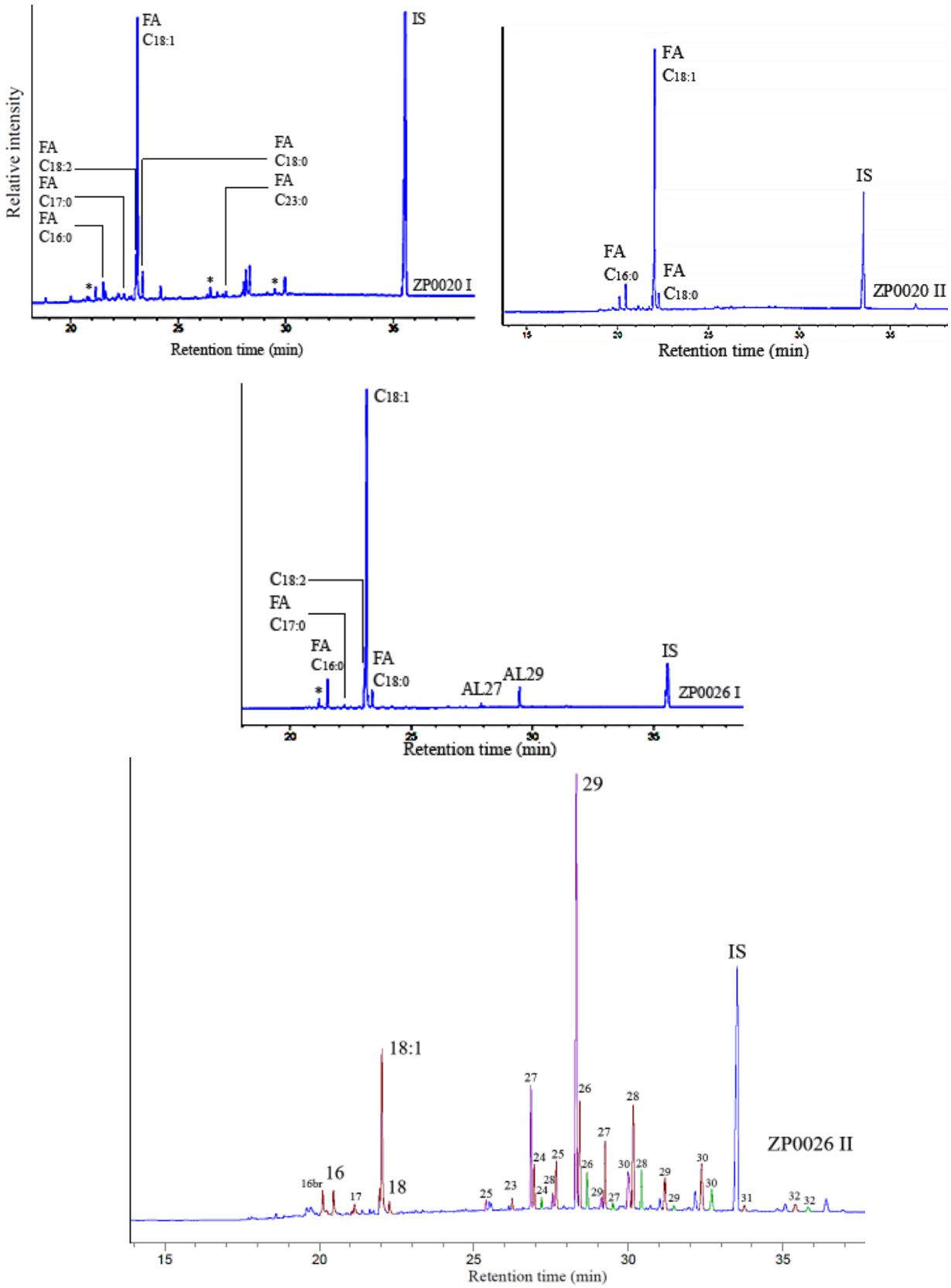

Figure 4-16 Partial gas chromatograms of the acidified methanol extracted from the same potsherd (ZP0020, ZP0026). The surface of II was cleaned deeper than the surface of I. Key: Fa Cx:y is the free fatty acids of carbon number X and Y degree of unsaturation, IS is the added internal standard (C_{34} *n*-alkane) and * denotes contamination. Red peaks denote fatty acids of X carbon number, green peaks represent *n*-alcohols of X carbon number, purple peaks are *n*-alkanes of X carbon number.

5 Conclusions and Future work

5.1 Conclusions

The organic residue analysis of archaeological potsherds from Zengpiyan leads to the following conclusions:

Overall, out of the 58 sherds investigated from Zengpiyan 33% yielded appreciable lipid residues with a mean concentration of 0.22 mg g^{-1}.

Most extracts were dominated by $C_{16:0}$ and $C_{18:0}$ fatty acids indicating the residues originated from animal fats. Moreover, they all contained odd-chain (particularly *n*-$C_{15:0}$ and *n*-$C_{17:0}$) and branched-chain fatty acids derived from ruminant animal fats. The abundance of biomarkers of rumen microflora in all the TLEs strongly suggested that hunting wild ruminant animals was widely practiced by this early Neolithic community.

The $\delta^{13}C$ and $\Delta^{13}C$ values of the major fatty acids ($C_{16:0}$ and $C_{18:0}$) of the animal fat residues in the archaeological pottery allowed differentiation between ruminant and non-ruminant carcass products processing. Of the animal-derived fats detected, ruminant carcass fats were predominately processed in the vessels (50%), followed by a lower rate of processing of non-ruminant animals (20%). The abundance of animal products processed within pottery vessels reflects the meat-based subsistence practice of the Zengpiyan people, confirmed by identifying substantial deer and pig remains identified in faunal assemblages.

Trends in $\delta^{13}C$ values of fatty acids extracted from potsherds also provide information regarding the contribution of C_3 and C_4 plant to the animals' diet. The wide range of $\delta^{13}C$ and $\Delta^{13}C$ values observed indicates various vegetation types existed in the surrounding environment or the animals were accessing different plant types due to seasonal changes or during animal migrations.

Plant biomarkers were present in 10 extracts, indicating the processing of mixtures of animal and plant commodities. The $\delta^{13}C$ values of long-chain fatty acids, ranging from –27.8‰ to -32.6‰, representing mainly C_3 plant origins. Where the LCFAs are present in low abundance compared to $C_{16:0}$ and $C_{18:0}$ their origin can be assumed to arise via direct routing from the animals plant diet to carcass fats. However, where LCFAs are the dominant compounds and where they occur with other plant biomarkers they are assumed to be residues of plant processing in pottery.

Diterpenoids were detected widely in the extracts but in low abundance. The exact reason for their presence is unknown but could relate to the processing of pine resins but could also be the residues of wood condensate from firing.

Aquatic resource exploitation should have taken place according to archaeological findings of abundant mollusc shells, however, probably due to low-temperature cooking or poor lipid preservation, no unequivocal aquatic biomarkers were identified. 9,10-dihydroxystearic acid was present in all TLEs, while only one extract contained the thermal produced biomarker C_{18} APAAs, and another the isoprenoid fatty acids, pristanic and phytanic acids. However, these compounds are not diagnostic of the processing of aquatic products since they can also be produced from terrestrial animal fats.

5.2 Recommendations for future work

This thesis describes the results of the first systematic absorbed organic residue investigations of archaeological potsherds from China. The results have shown that lipid residue survive in hunter gatherer pottery and provide a first glimpse of human diet for at Neolithic Zengpiyan. However, the number of sherds studied is quite small hence greater insights would be gained from increasing the number of sherds.

One surprising result is the lack of biomarker evidence for aquatic resource processing given the large number of mollusk shells recovered at the site. Further evidence my come from analyses of larger numbers of sherds.

Given the large number of animals bones preserved at the site there would be some value in investigating the relationships between the stable isotopes of the deer and pig bones and the animal fats in the pottery. This would provide further corroboration of the diversity of plants in the ecosystems of Zengpiyan and the feeding behaviors of the animals.

The specific objective of this project was to provide preliminary results for organic residues in prehistoric Chinese pottery. As mentioned before, China possesses thousands of archaeological sites, almost none of them have systematically applied ORA. The results obtained from the Zengpiyan potsherds show that considerable potential exists for expanding such investigations to address different chronological and regional questions in Chinese prehistory, integrating organic residue results with other archaeological information to improve the understanding of early diet and subsistence, and development of agriculture. For

example, archaeological and archaeobotanical evidence suggest plant cultivation in Neolithic China started from two major river regions, the Yangzi River and the Yellow River, based on the substantial discovery of plant remains and cultivation artefacts. A larger geographical sampling programme and organic residues analysis of pottery would help elucidate the spatiotemporal extent of plant gathering/cultivation and processing. Specific lipid biomarkers can be used as proxy for certain plant sources, e.g. the triterpenoid miliacin could be used as a biomarker for broomcorn millet processing in bronze age pottery from China (Heron *et al.,* 2016).

The dating of Zengpiyan remains highly controversial. Compound-specific radiocarbon dating approach is a novel method to date lipids absorbed into the unglazed vessel matrix. If lipid concentrations are present at the required level, this approach become a possibility for resolving dating uncertainties. At Zengpiyan the ^{14}C ages are mainly derived from charcoal, however, the radiocarbon ages from charcoals are easily overestimated due to re-use, i.e. "the old wood effect". Compound-specific radiocarbon dating of pottery lipid residues offers a unique way of accurately dating archaeological potsherds. One of the advantages is that lipids will have "young" ages due to the short life-time of use of pots, however, the technique requires > 200 ug/g of C per compound for accurate dates to be obtained. The results obtained from Zengpiyan show compound-specific dating of the pottery is possible and work in progress to test this. Clearly, this compound-specific dating approach would help to resolve questions surrounding the dates of early pottery in China.

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Appendix 1

Summary of lipid compositions detected in pottery vessels from Zengpiyan

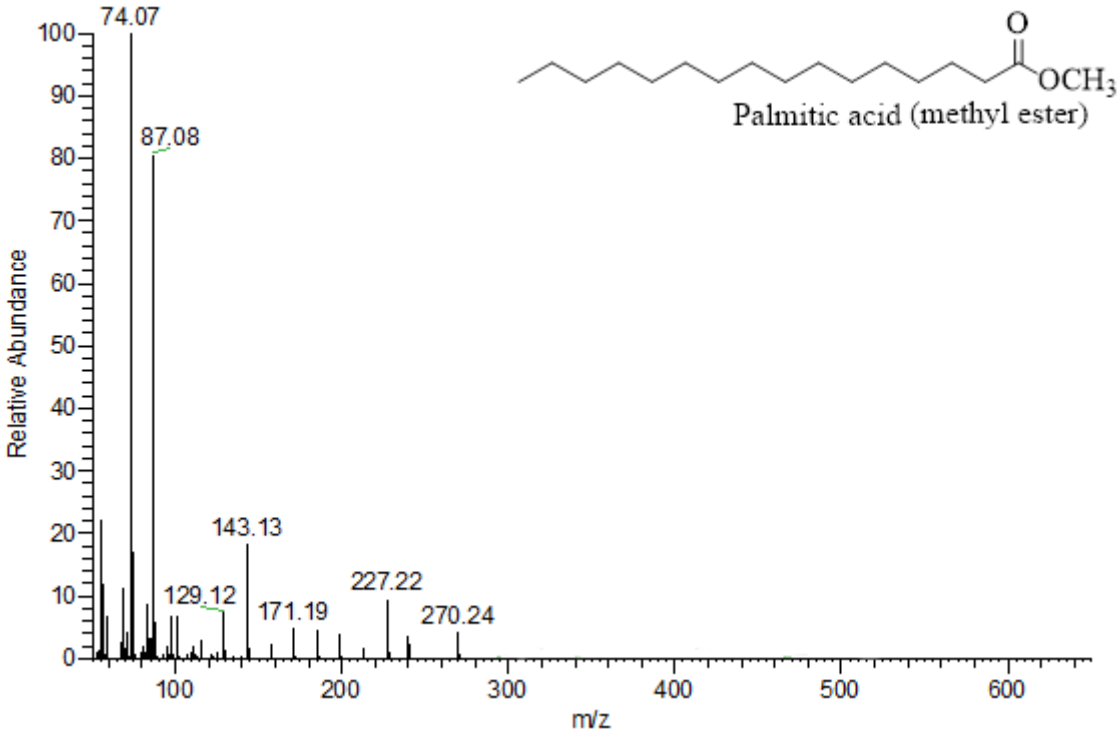
Sample	Lipid Concentration ($\mu g\ g^{-1}$)	FA	Alcohols	Alkanes	Terpenoid	Ketones	Other	$\delta^{13}C_{16:0}$ (‰)	$\delta^{13}C_{18:0}$ (‰)	$\Delta^{13}C$ (‰)	Origin of animal fats	Origin of plant-derived lipids
ZP0001	682	C14-C33	C24-C30, C32	C24-C27, C29	Pine; Benzoic; 2-methyl-benzoic	-	Glycerol; cedrene	-27.5	-29.2	-1.7	Mixture animal/plant fats	C_3 plants
ZP0002	-	-	-	-	-	-	-	-	-	-	-	-
ZP0003	-	-	-	-	-	-	-	-	-	-	-	-
ZP0004	6	-	-	-	-	-	-	-	-	-	-	-
ZP0005	10	-	-	-	-	-	-	-	-	-	-	-
ZP0006	11	-	-	-	-	-	-	-	-	-	-	-
ZP0007	7	-	-	-	-	-	-	-	-	-	-	-
ZP0008	-	-	-	-	-	-	-	-	-	-	-	-
ZP0009	1	-	-	-	-	-	-	-	-	-	-	-
ZP0010	68	C16-C32	C22-30, C32	C25, C27	Pine; Benzoic; 2-methyl-benzoic	-	cedrene	-20.2	-23.6	-3.6	Mixture animal/plant fats	Mixture C_3/ C_4 plants
ZP0011	1385	C14-C32	C20-C30, C32	C23-C27, C29	-	-	Glycerol; cedrene	-27.6	-27.2	0.6	-	C_3 plants
ZP0012	-	-	-	-	-	-		-	-	-	-	-
ZP0013	60	C14-C26	-	-	Pine; Benzoic	-	Cholesterol	-24.5	-27.5	-3.0	RA/RDA	-
ZP0014	5	-	-	-	-	-	-	-	-	-	-	-
ZP0015	-	-	-	-	-	-	-	-	-	-	-	-
ZP0016	-	-	-	-	-	-	-	-	-	-	-	-
ZP0017	-	-	-	-	-	-	-	-	-	-	-	-
ZP0018	2	-	-	-	-	-	-	-	-	-	-	-

Sample	Lipid Concentration ($\mu g\ g^{-1}$)	FA	Alcohols	Alkanes	Terpenoid	Ketones	Other	$\delta^{13}C_{16:0}$ (‰)	$\delta^{13}C_{18:0}$ (‰)	$\Delta^{13}C$ (‰)	Animal assignment	Plant assignment
ZP0019	2	-	-	-	-	-	-	-	-	-	-	-
ZP0020	24	C14, C16-C18, C23	-	-	Pine; Benzoic	-	Cholesterol?	-15.3	-13.7	1.6	NRA	-
ZP0021	3	-	-	-	-	-	-	-	-	-	-	-
ZP0022	15	-	-	-	-	-	-	-	-	-	-	-
ZP0023	4	-	-	-	-	-	-	-	-	-	-	-
ZP0024	14	-	-	-	-	-	-	-	-	-	-	-
ZP0025	4	-	-	-	-	-	-	-	-	-	-	-
ZP0026	94	C16-C18, C23-C32	C24, C26-C30, C32	C25-C30	Pine; Benzoic	-	Cholesterol?	-20.5	-	-	-	-
ZP0027	21	-	-	-	-	-	-	-26.4	-27.8	-1.4	RA	-
ZP0028	30	C15-C18, C24	-	-	Pine		Cholesterol	-22.0	-20.6	1.4	NRA	-
ZP0029	9	C16-C28	-	-	Pine	-	-	-26.0	-28.6	-2.6	RA	-
ZP0030	6	-						-	-	-	-	-
ZP0031	37	C15-C30, C32	C26, C28, C30	-	Pine	-	Cholesterol?	-28.4	-31.3	-2.9	RA/RD	-
ZP0032	23	C16-18, C24	-	-	Pine	-	Cholesterol	-	-20.6	-	-	-
ZP0033	135	C14-C30, C32	C26, C28, C30	-	Pine; Benzoic	-	C_{18} APAAs	-27.4	-29.3	-1.8	RA	-
ZPB0001	2	-	-					-	-	-	-	-
ZPB0002	-	C16, C18, C20, C24	-	-	-	-	-	-25.6	-27.4	-1.8	RA	-
ZPB0003	18	C16-C24, C26	-	-	Pine		Cholesterol, Pristanic, phytanic acids	-22.9	-25.2	-2.3	RA	-
ZPB0004	-	-						-	-	-	-	-
ZPB0005	4	-						-	-	-	-	-
ZPB0006	13	C16, C18, C20-C30	-	C23, C25, C27, C30	Pine		Cholesterol	-26.2	-33.3	-7.1	RDA	-

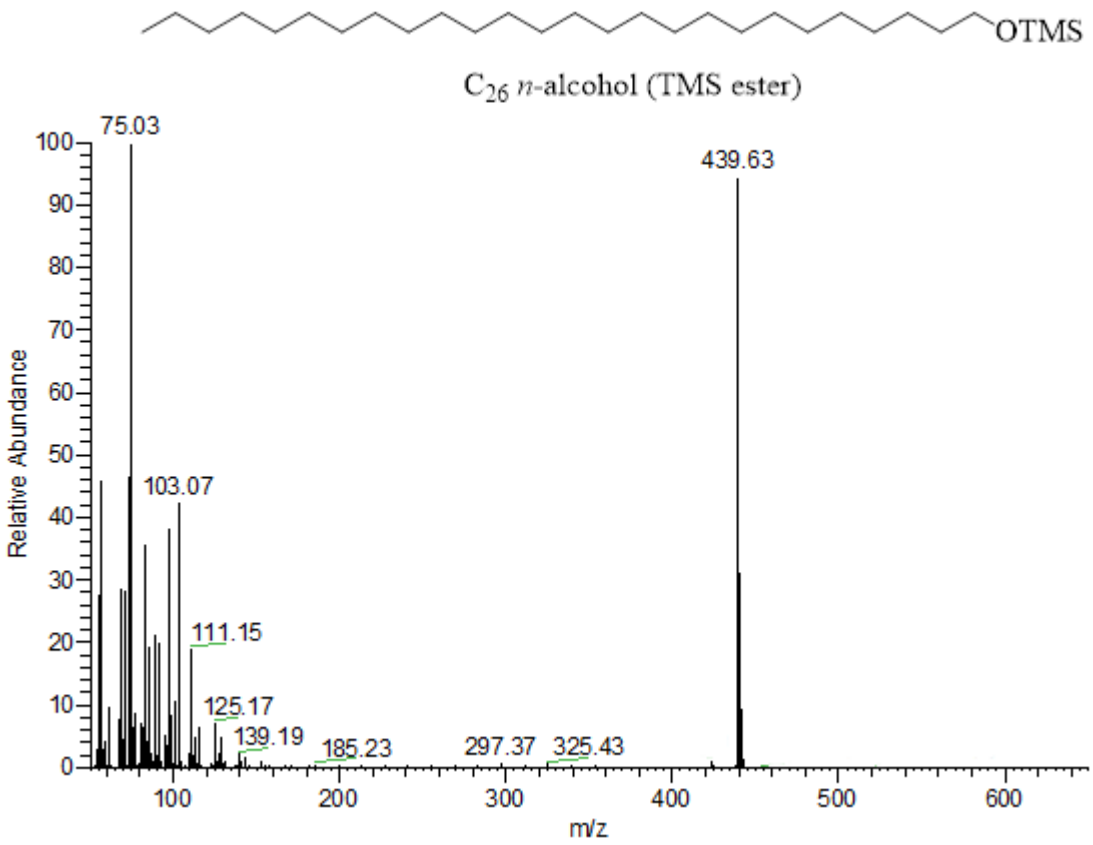
Sample	Lipid Concentration ($\mu g\ g^{-1}$)	FA	Alcohols	Alkanes	Terpenoid	Ketones	Other	$\delta^{13}C_{16:0}$ (‰)	$\delta^{13}C_{18:0}$ (‰)	$\Delta^{13}C$ (‰)	Animal assignment	Plant assignment
ZPB0007	-	-						-	-	-	-	-
ZPD0001	-	-						-	-	-	-	-
ZPD0002	-	-						-	-	-	-	-
ZPD0003	7							-	-	-	-	-
ZPD0004	9	C16, C18, C20-C32	-	-	Pine		-	-	-	-	-	-
ZPD0005	37	C16-C27	-	-	Pine	-	-	-28.3	-29.8	-1.6	RA	-
ZPD0006	-	-						-	-	-	-	-
ZPD0007	9											-
ZPD0008	-							-	-	-	-	-
ZPD0009	-	-						-	-	-	-	-
ZPD0010	-	-						-	-	-	-	-
ZPD0011	-	-						-	-	-	-	-
ZPD0012	19											-
ZPD0013	81	C16-C34	C24, C26, C28, C30, C32	-	Pine	-	Cholesterol	-27.2	-29.1	-1.9	Mixture animal and plant fats	C_3 plants
ZPD0014	183	C15-C32	-	-	Pine	-	Cholesterol?	-22.7	-21.7	1.0	NRA	-
ZPD0015	178	C15-C30	-	-	Pine	-	Cholesterol	-27.2	-27.0	0.2	NRA/RA	
ZPD0016	-	-	-	-	-	-	-	-	-	-	-	--
ZPD0017	120	C16-C30	-	-	Pine?	-	-	-28.1	-27.7	0.4	NRA	-
ZPD0018	5	C16-C18, C24, C26, C28	-	-	Pine	-	Cholesterol	-25.9	-26.8	-0.9	RA	-

Appendix 2

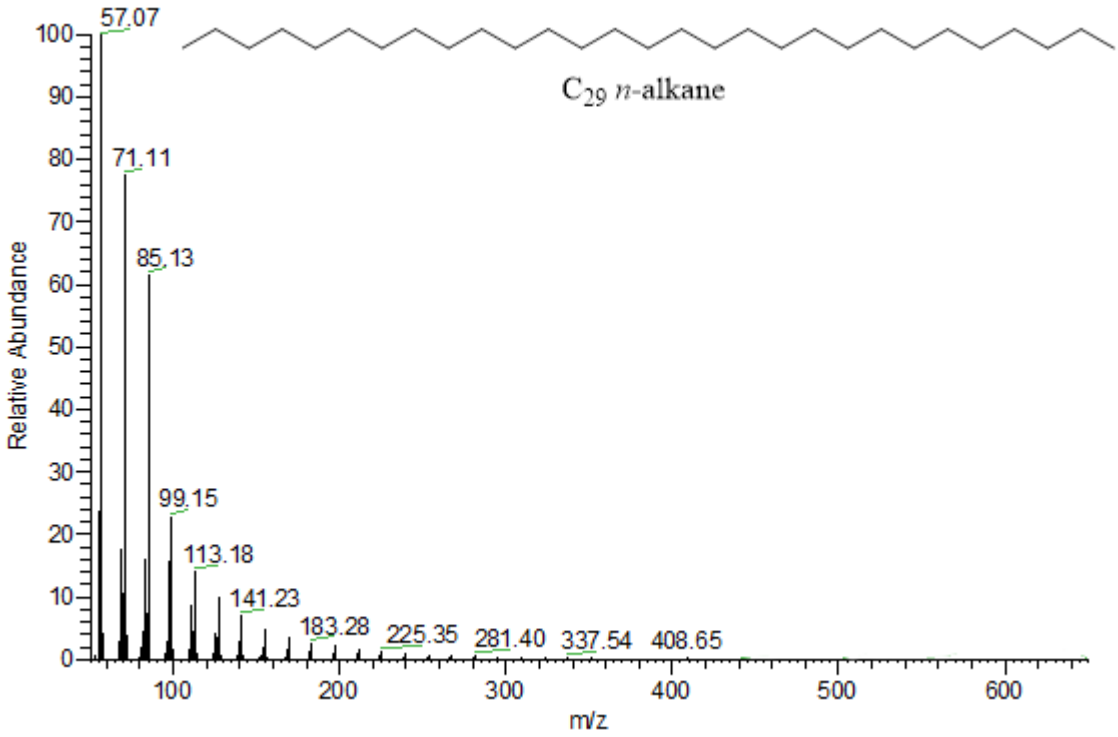
Mass spectra of detected compounds



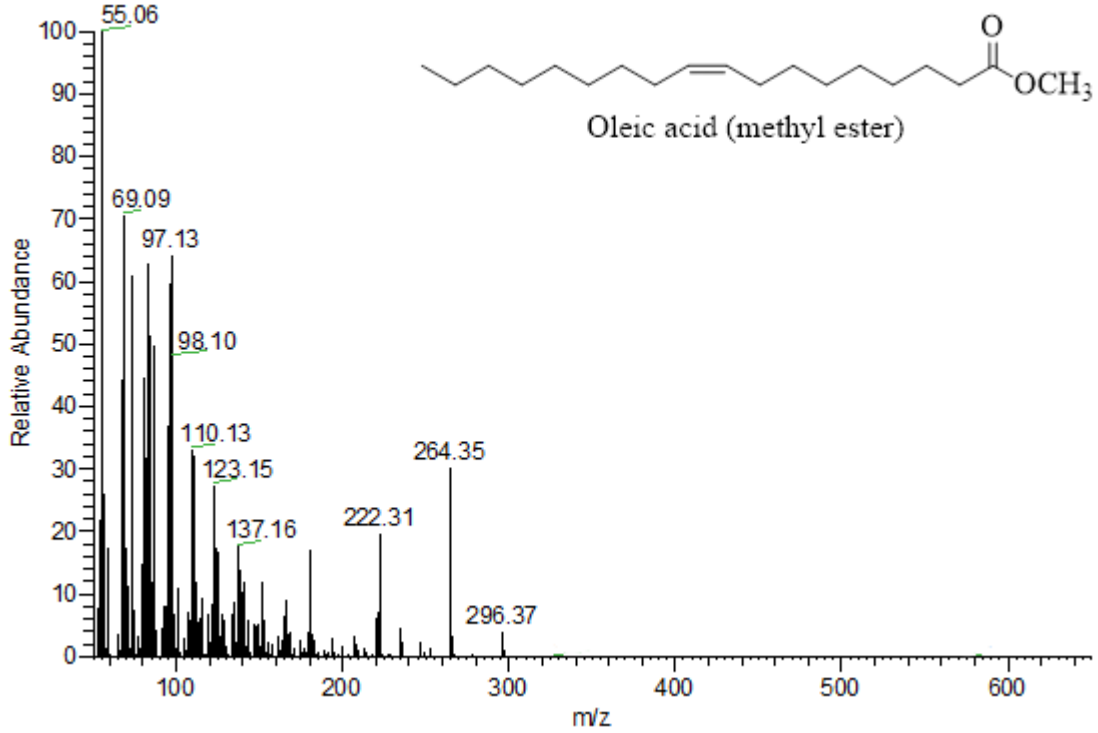
A2.1 Mass spectra of palmitic acid ($C_{16:0}$ fatty acid; $M^{+\cdot}$ 270).



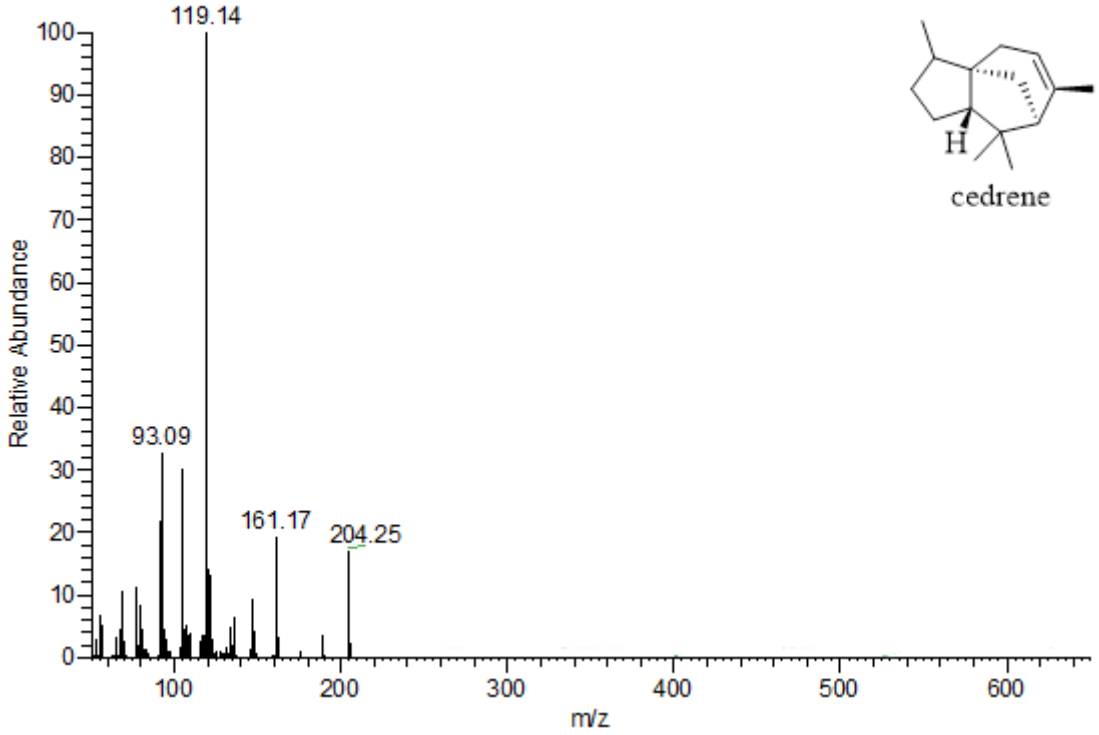
A2.2 Mass spectra of C_{26} *n*-alcohol ($M^{+\cdot}$ 439).



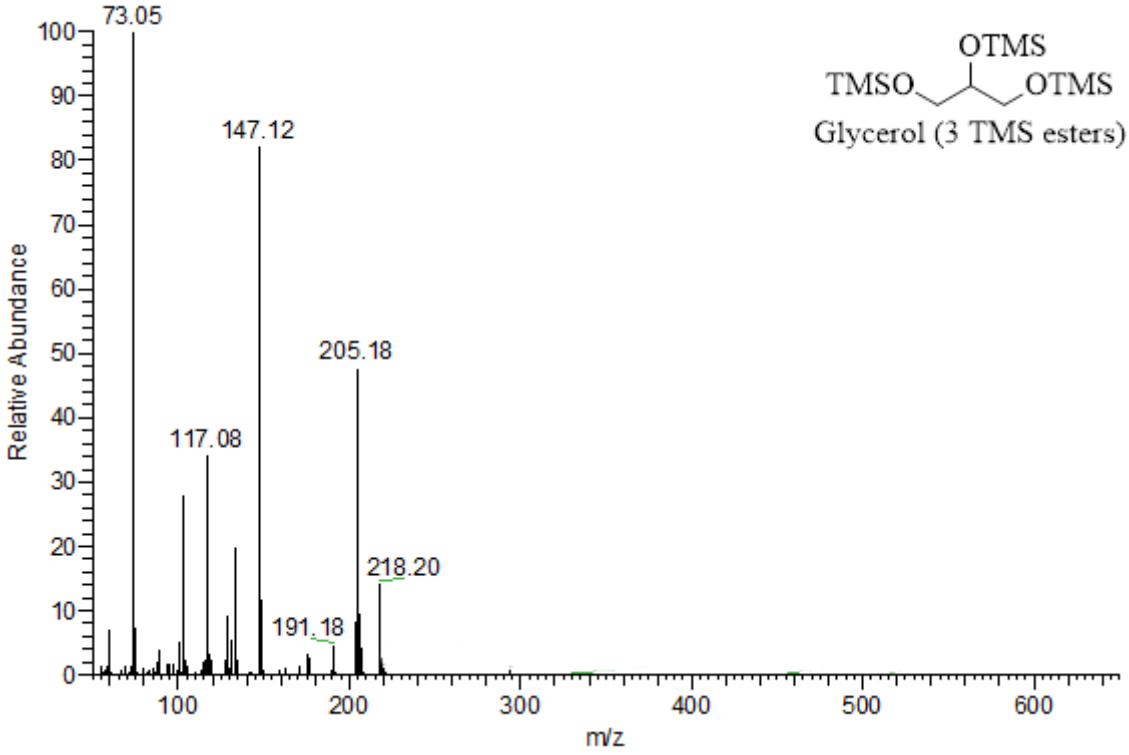
A2.3 Mass spectra of C_{29} *n*-alkane ($M^{+\cdot}$ 408).



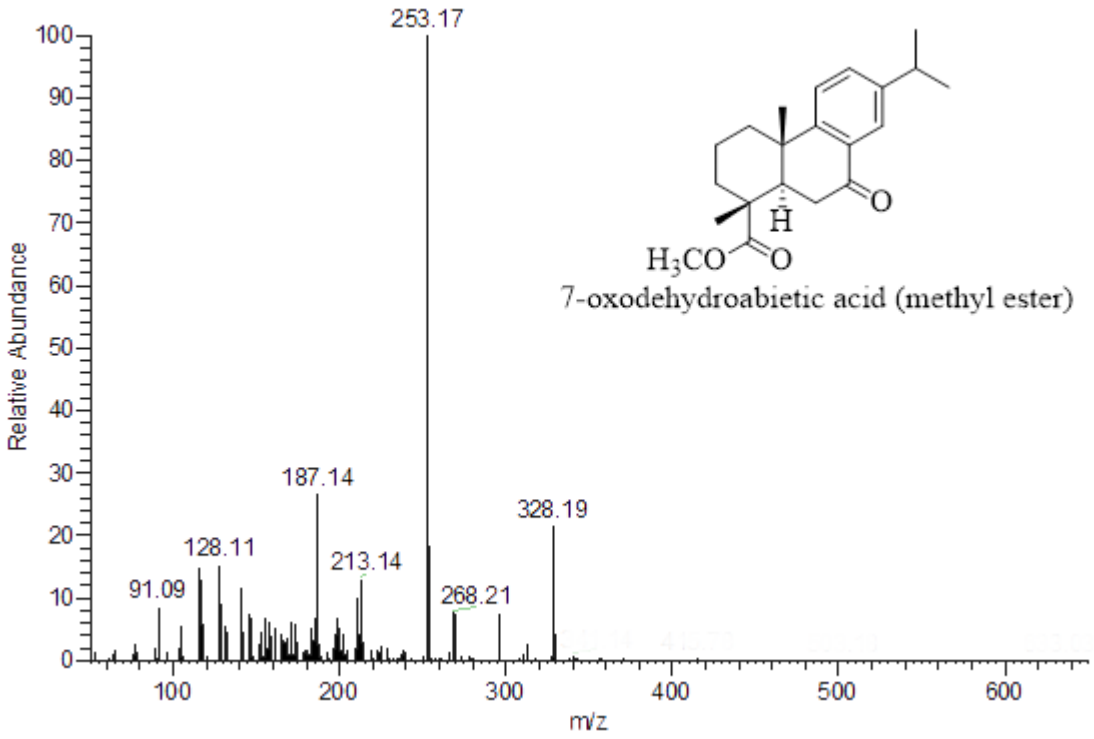
A2.4 Mass spectra of oleic acid (unsaturated $C_{18:1}$; $M^{+\cdot}$ 296).



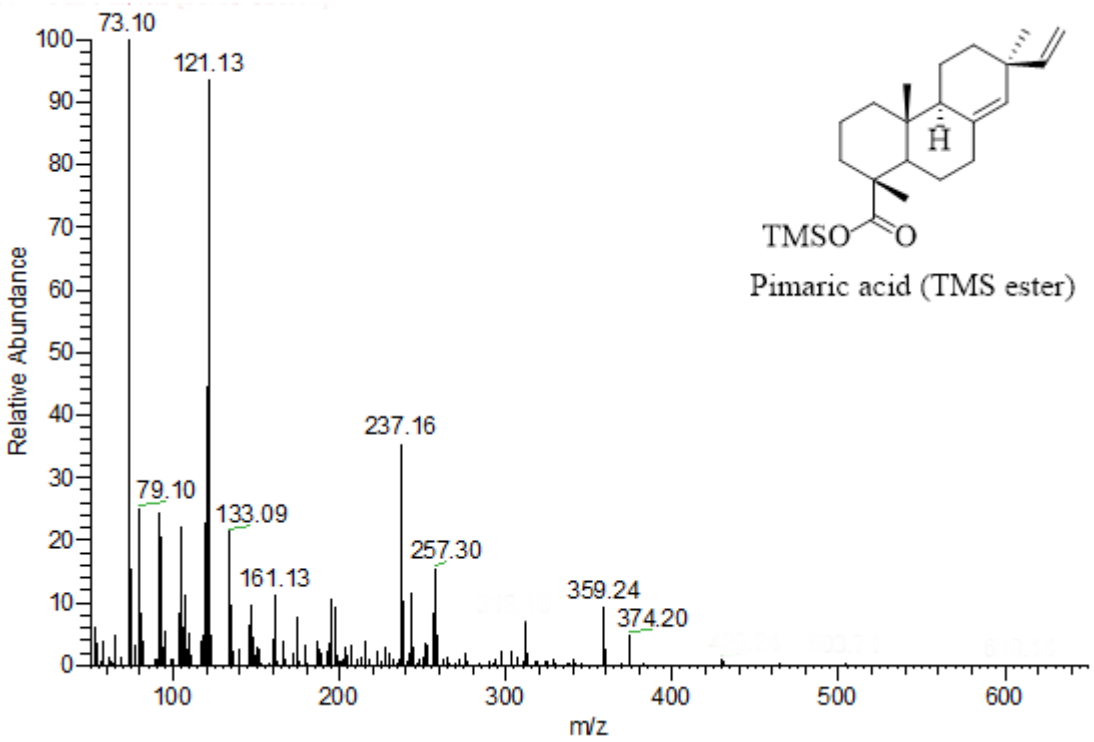
A2.5 Mass spectra of α-cedrene ($M^{+\cdot}$ 204).



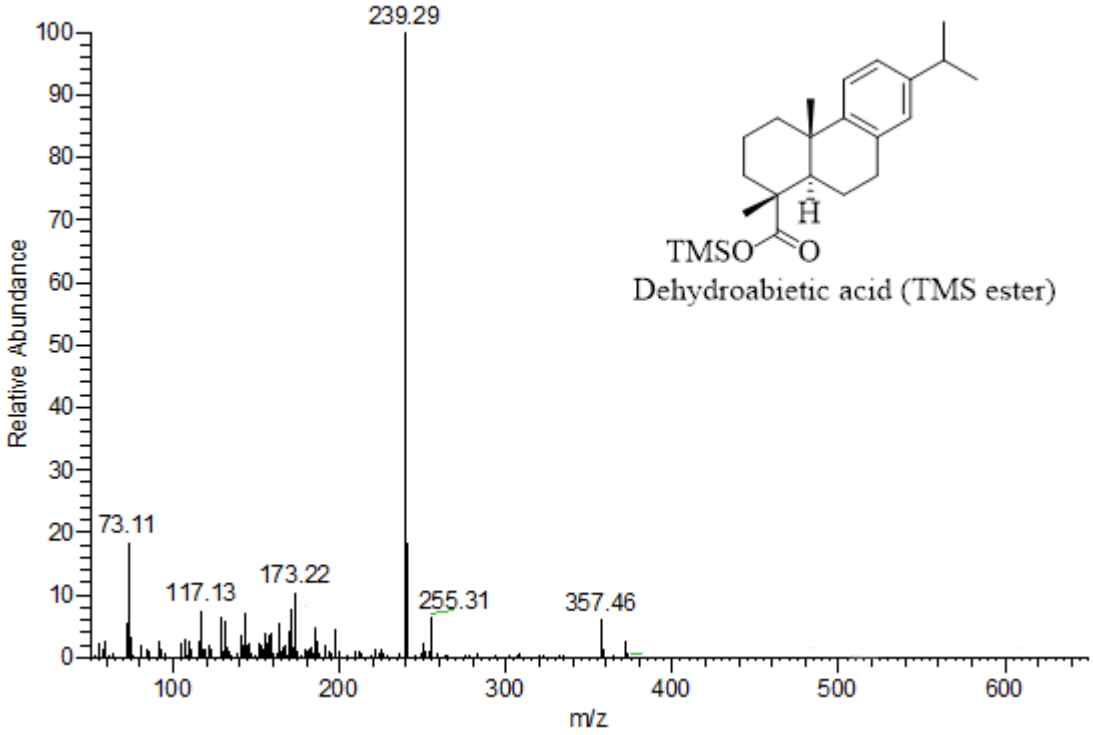
A2.6 Mass spectra of glycerol ($M^{+\cdot}$ 218).



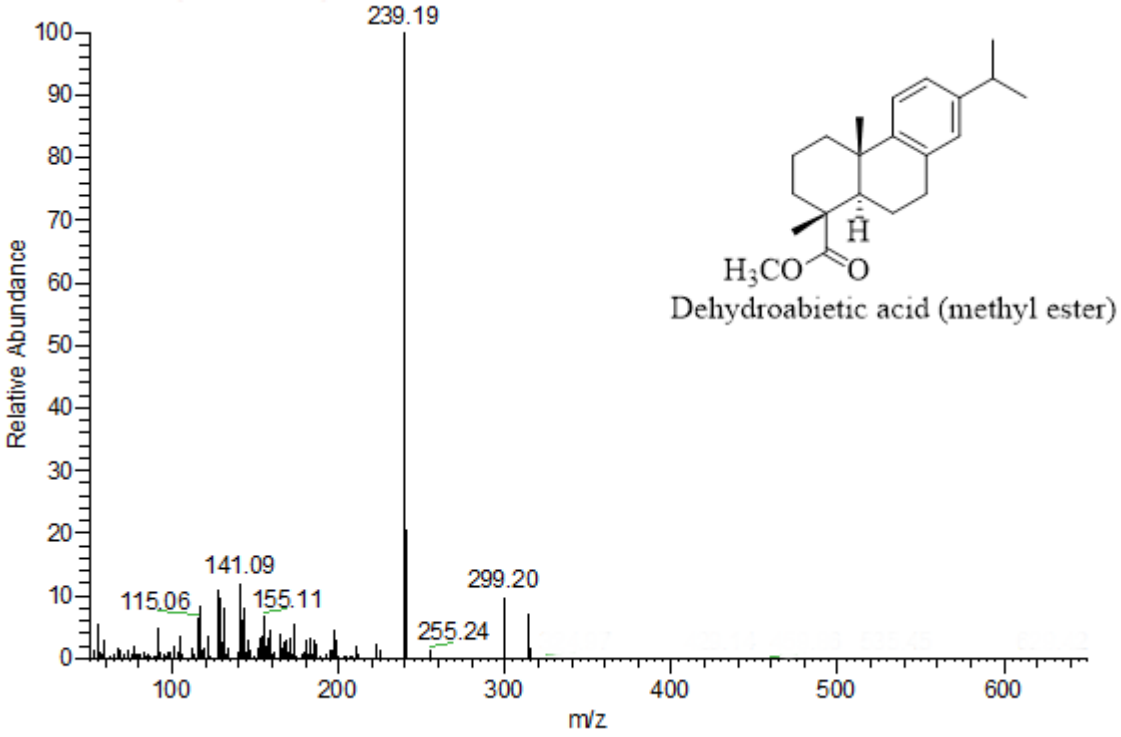
A2.7 Mass spectra of 7-oxodehysroabietic acid ($M^{+\cdot}$ 328).



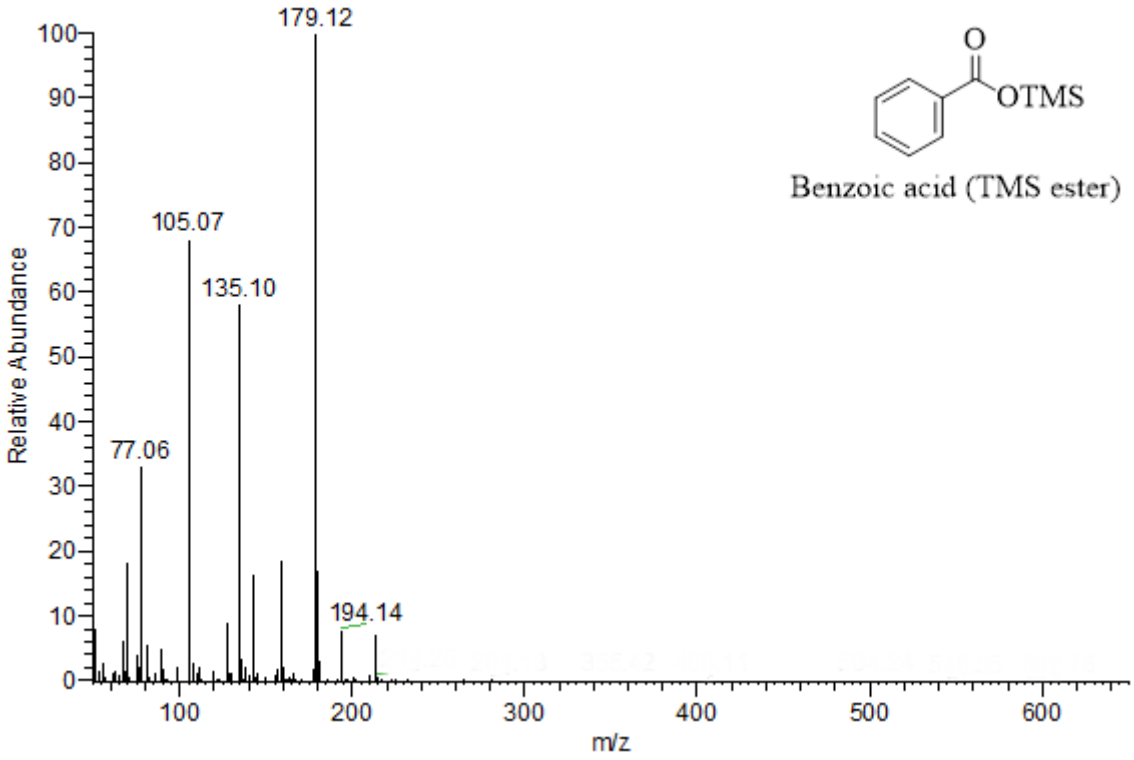
A2.8 Mass spectra of pimaric acid ($M^{+\cdot}$ 374).



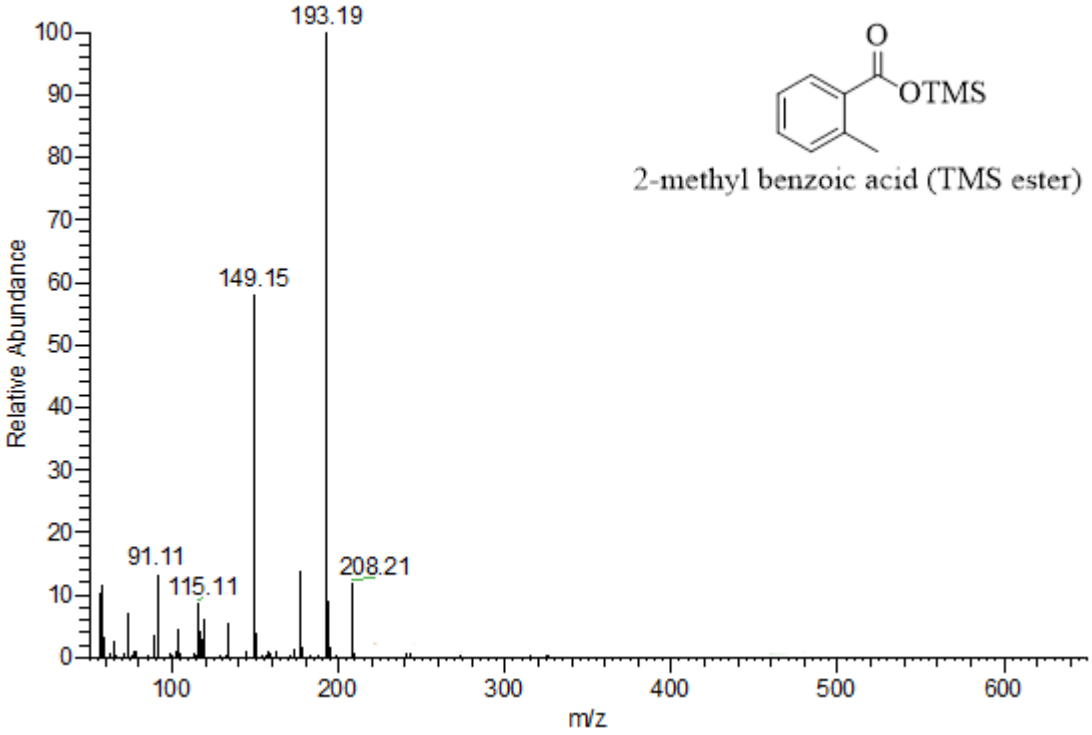
A2.9 Mass spectra of dehydroabietic acid ($M^{+\cdot}$ 357).



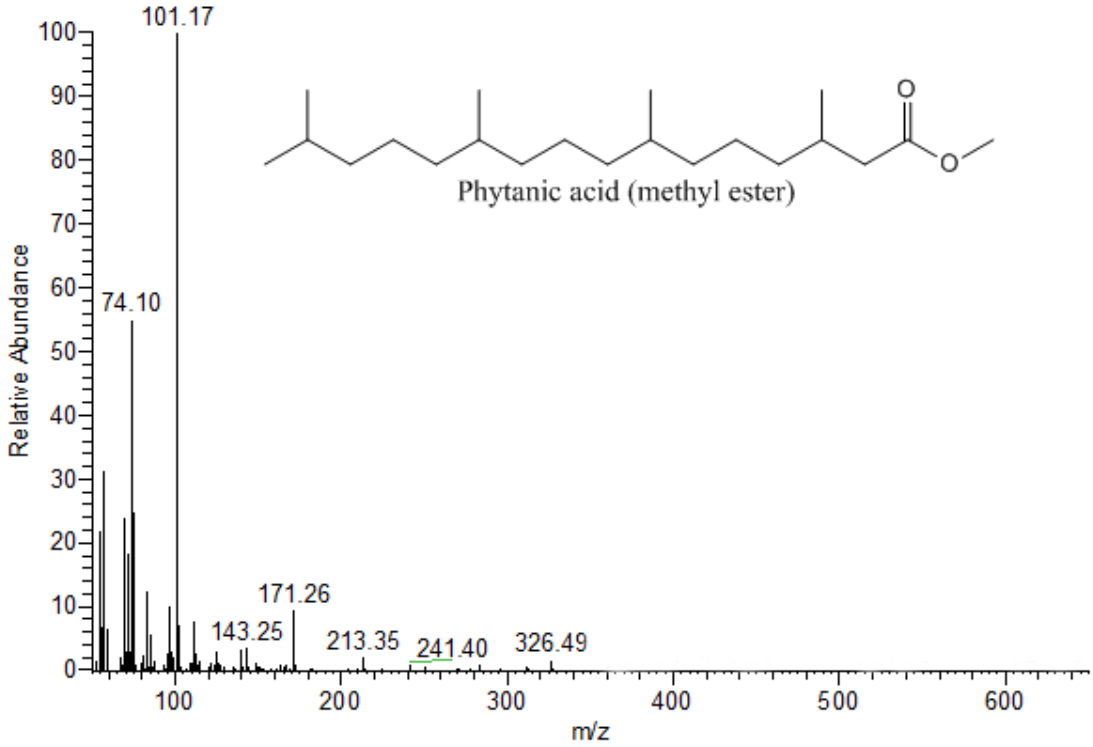
A2.10 Mass spectra of dehydroabietic acid ($M^{+\cdot}$ 314).



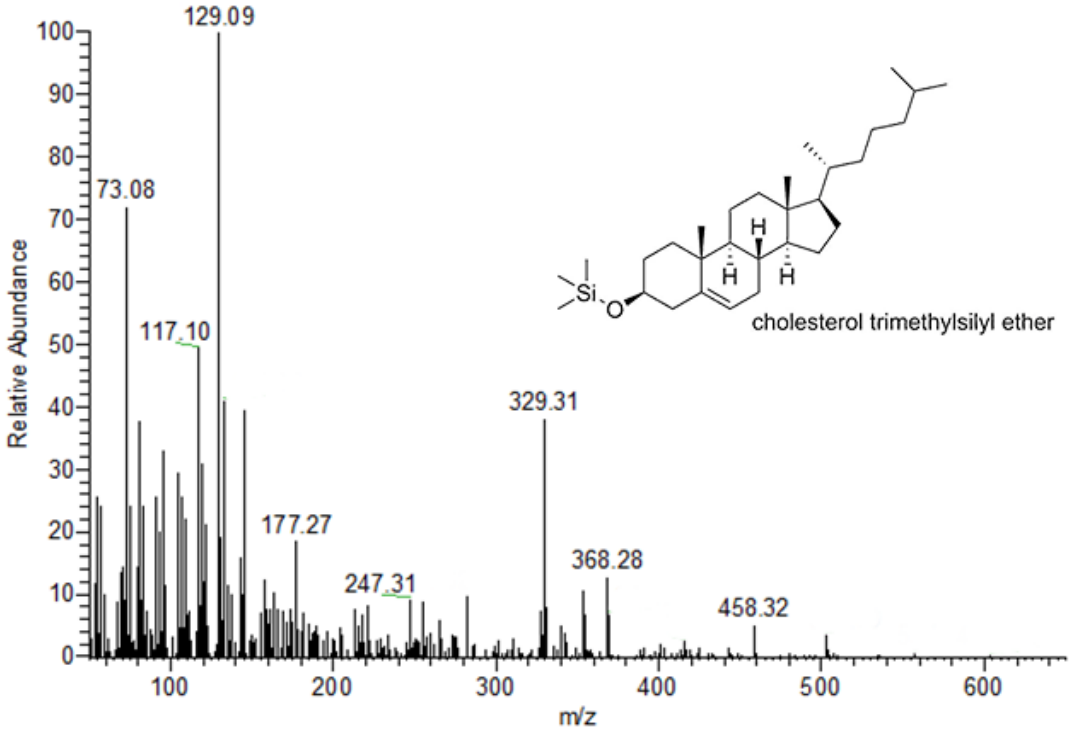
A2.11 Mass spectra of benzoic acid ($M^{+\cdot}$ 193).



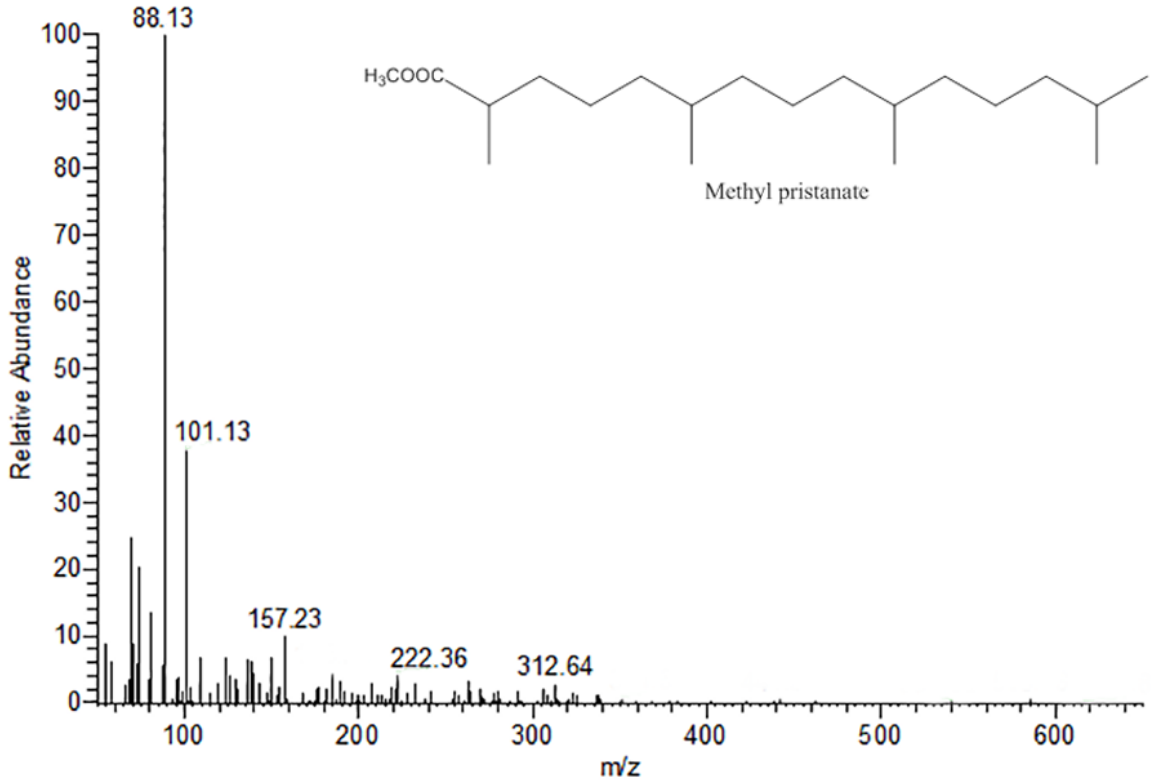
A2.12 Mass spectra of 2-methyl-benzoic acid ($M^{+\cdot}$ 208).



A2.13 Mass spectra of phytanic acid ($M^{+\cdot}$ 326).



A2.14 Mass spectra of cholesterol ($M^{+\cdot}$ 458).



A2.15 Mass spectra of pristanic acid ($M^{+\cdot}$ 312).